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Air Quality Criteria for Lead

Volume I of II

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Air Quality Criteria for Lead

Volume I

National Center for Environmental Assessment-RTP Division
Office of Research and Development
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PREFACE

National Ambient Air Quality Standards (NAAQS) are promulgated by the United States Environmental Protection Agency (EPA) to meet requirements set forth in Sections 108 and 109 of the U.S. Clean Air Act. Those two Clean Air Act sections require the EPA Administrator (1) to list widespread air pollutants that reasonably may be expected to endanger public health or welfare; (2) to issue air quality criteria for them that assess the latest available scientific information on nature and effects of ambient exposure to them; (3) to set "primary" NAAQS to protect human health with adequate margin of safety and to set "secondary" NAAQS to protect against welfare effects (e.g., effects on vegetation, ecosystems, visibility, climate, manmade materials, etc); and (5) to periodically review and revise, as appropriate, the criteria and NAAQS for a given listed pollutant or class of pollutants.

Lead (Pb) was first listed in the mid-1970's as a "criteria air pollutant" requiring NAAQS regulation. The scientific information pertinent to Pb NAAQS development available at the time was assessed in the EPA document *Air Quality Criteria for Lead*; published in 1977. Based on the scientific assessments contained in that 1977 lead air quality criteria document (1977 Lead AQCD), EPA established a 1.5 $\mu g/m^3$ (maximum quarterly calendar average) Pb NAAQS in 1978.

To meet Clean Air Act requirements noted above for periodic review of criteria and NAAQS, new scientific information published since the 1977 Lead AQCD was later assessed in a revised Lead AQCD and Addendum published in 1986 and in a Supplement to the 1986 AQCD/Addendum published by EPA in 1990. A 1990 Lead Staff Paper, prepared by EPA's Office of Air Quality Planning and Standards (OPQPS), drew upon key findings and conclusions from the 1986 Lead AQCD/Addendum and 1990 Supplement (as well as other OAQPS-sponsored lead exposure/risk analyses) in posing options for the EPA Administrator to consider with regard to possible revision of the Pb NAAQS. However, EPA chose not to revise the Pb NAAQS at that time. Rather, as part of implementing a broad 1991 U.S. EPA Strategy for Reducing Lead Exposure, the Agency focused primarily on regulatory and remedial clean-up efforts to reduce Pb exposure from a variety of non-air sources that posed more extensive public health risks, as well as other actions to reduce air emissions.

The purpose of this revised Lead AQCD is to critically assess the latest scientific information that has become available since the literature assessed in the 1986 Lead

AQCD/Addendum and 1990 Supplement, with the main focus being on pertinent new information useful in evaluating health and environmental effects of ambient air lead exposures. This includes discussion in this document of information regarding: the nature, sources, distribution, measurement, and concentrations of lead in the environment; multimedia lead exposure (via air, food, water, etc.) and biokinetic modeling of contributions of such exposures to concentrations of lead in brain, kidney, and other tissues (e.g., blood and bone concentrations, as key indices of lead exposure).; characterization of lead health effects and associated exposure-response relationships; and delineation of environmental (ecological) effects of lead. This final version of the revised Lead AQCD mainly assesses pertinent literature published or accepted for publication through December 2005.

The First External Review Draft (dated December 2005) of the revised Lead AQCD underwent public comment and was reviewed by the Clean Air Scientific Advisory Committee (CASAC) at a public meeting held in Durham, NC on February 28-March 1, 2006. The public comments and CASAC recommendations received were taken into account in making appropriate revisions and incorporating them into a Second External Review Draft (dated May, 2006) which was released for further public comment and CASAC review at a public meeting held June 28-29, 2006. In addition, still further revised drafts of the Integrative Synthesis chapter and the Executive Summary were then issued and discussed during an August 15, 2006 CASAC teleconference call. Public comments and CASAC advice received on these latter materials, as well as Second External Review Draft materials, were taken into account in making and incorporating further revisions into this final version of this Lead AQCD, which is being issued to meet an October 1, 2006 court-ordered deadline. Evaluations contained in the present document provide inputs to an associated Lead Staff Paper prepared by EPA's Office of Air Quality Planning and Standards (OAQPS), which poses options for consideration by the EPA Administrator with regard to proposal and, ultimately, promulgation of decisions on potential retention or revision, as appropriate, of the current Pb NAAQS.

Preparation of this document has been coordinated by staff of EPA's National Center for Environmental Assessment in Research Triangle Park (NCEA-RTP). NCEA-RTP scientific staff, together with experts from academia, contributed to writing of document chapters. Earlier drafts of document materials were reviewed by scientists from other EPA units and by non-EPA experts in several public peer consultation workshops held by EPA in July/August 2005.

NCEA acknowledges the valuable contributions provided by authors, contributors, and reviewers and the diligence of its staff and contractors in the preparation of this document. The constructive comments provided by public commenters and CASAC that served as valuable inputs contributing to improved scientific and editorial quality of the document are also acknowledged by NCEA.

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Abbreviations and Acronyms

αFGF	α-fibroblast growth factor
AA	arachidonic acid; atomic absorption
AAL	active avoidance learning
AALM	All Ages Lead Model
AAS	atomic absorption spectroscopy
ACBP	Achenbach Child Behavior Profile
ACE	angiotensin converting enzyme
AChE	acetylcholinesterase
ACSL	Advanced Continuous Simulation Language
ADCC	antibody-dependent cellular cytotoxicity
ADHD	attention deficit/hyperactivity disorder
ADP	adenosine diphosphate
AF	absorption fraction
A horizon	uppermost layer of soil (litter and humus)
AHR	aryl hydrocarbon receptor
ALA	δ-aminolevulinic acid; 5-aminolevulinic acid
ALAD	δ-aminolevulinic acid dehydratase
ALAS	aminolevulinic acid synthase
ALAU	δ-aminolevulinic acid dehydratase
ALM	Adult Lead Methodology
ALS	amyotrophic lateral sclerosis
ALT	alanine aminotransferase; alanine transferase
AMD	activity mean diameter
AMP	adenosine monophosphate
ANF	atrial natriuretic factor
ANOVA	analysis of variance
AP	alkaline phosphatase
AP-1	activator protein-1
APE	apurinic endonuclease
ApoE	apolipoprotein E
APP	amyloid precursor protein
AQCD	Air Quality Criteria Document

ASA	arylsulfatase
AST	aspartate aminotransferase
ASV	anode stripping voltammetry
ATP	adenosine triphosphate
ATP1A2	sodium-potassium adenosine triphosphase α2
ATPase	adenosine triphosphate synthase
ATSDR	Agency for Toxic Substances and Disease Research
ATV	all-terrain vehicle
AVS	acid volatile sulfide
AWQC	ambient water quality criteria
β	beta-coefficient; slope of an equation
βFGF	β-fibroblast growth factor
6-β-OH-cortisol	6-β-hydroxycortisol
BAEP	brainstem auditory-evoked potentials
BBB	blood-brain barrier
B cell	B lymphocyte
BCF	bioconcentration factor
BDNF	brain-derived neurotrophic factor
BLL	blood lead level
BLM	biotic ligand model
BMDM	bone marrow-derived macrophages
BMI	body mass index
BMP	bone morphogenic protein
BRHS	British Regional Heart Study
BSID	Bayley Scales of Infant Development
BTQ	Boston Teacher Questionnaire
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
^{45}Ca, ^{47}Ca	calcium-45 and -47 radionuclides
CA1	cornu ammonis 1 region of hippocampus
CA3	cornu ammonis 3 region of hippocampus
CAA	Clean Air Act
Ca-ATPase	calcium-dependent adenosine triphosphatase

$^{43}CaCl_2$	calcium-43 radionuclide-labeled calcium chloride
$CaCO_3$	calcium carbonate
CaEDTA	calcium disodium ethylenediaminetetraacetic acid
CAL	calcitonin
CAMKII	calcium/calmodulin-dependent protein kinase
cAMP	cyclic adenosinemonophosphate
$CaNa_2$ EDTA	calcium disodium ethylenediaminetetraacetic acid
CANTAB	Cambridge Neuropsychological Testing Automated Battery
CAP	criteria air pollutant
$Ca_{10}(PO_4)_6(OH)_2$	hydroxyapatite
CASAC	Clean Air Scientific Advisory Committee
CBCL	Achenbach Child Behavior Checklist
CCE	Coordination Center for Effects
Cd	cadmium
CD	Sprague-Dawley CD (rat)
CDC	Centers for Disease Control and Prevention
CERR	Consolidated Emissions Reporting Rule
CESD, CES-D	Center for Epidemiologic Studies Depression (scale)
cGMP	cyclic guanosine-3',5'-monophosphate; cyclic guanylylmonophosphate
CI	confidence interval
CKD	chronic kidney disease
CLRTAP	Convention on Long-range Transboundary of Air Pollution
CMI	cell-mediated immunity
CNS	central nervous system
CO_2	carbon dioxide
ConA	concanavalin A
COX-2	cyclooxygenase-2
CP	coproporphyrin
CPT	current perception threshold
CRAC	calcium release activated calcium reflux
CREB	cyclic-AMP response element binding protein
CRI	chronic renal insufficiency
CSF	cerebrospinal fluid

CSF-1	colony-stimulating factor-1
CTL	cytotoxic T lymphocyte
CuZnSOD	copper and zinc-dependent superoxide dismutase
CWA	Clean Water Act
CYP	cytochrome (e.g., CYP1A, CYP-2A6, CYP3A4, CYP450)
DA	dopamine; dopaminergic
DET	diffusive equilibrium thin films
DFS	decayed or filled surfaces, permanent teeth
dfs	covariate-adjusted number of caries
DGT	diffusive gradient thin films
DiAL	dialkyllead
DMEM	Dulbecco's Modified Eagle Medium
DMFS	decayed, missing, or filled surfaces, permanent teeth
DMSA	2,3-dimercaptosuccinic acid; dimethyl succinic acid
DMTU	dimethyl thio urea
DNA	deoxyribonucleic acid
DNTC	diffuse neurofibrillary tangles with calcification
DOC	dissolved organic carbon
DOM	dissolved organic matter
DOS	Disk Operating System
DPH	1, 6-diphenyl-1,3,5-hexatriene
DRL	differential reinforcement of low rate (schedule)
DSA	delayed spatial alternation
DTC	dithiocarbamate
DTH	delayed type hypersensitivity
E	embryonic day; epinephrine
E_2	estradiol
EBE	early biological effect
EC	coronary endothelial (cells)
EC_{50}	effect concentration for 50% of test population
ECF	extracellular fluid
Eco-SSL	ecological soil screening level
EDRF	endothelium-derived relaxing factor
EDTA	ethylenediaminetetraacetic acid

EEDQ	*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinone
EEG	electroencephalogram
EGF	epidermal growth factor
EGTA	ethyleneglycoltetraacetic acid
eNOS	endothelial nitric oxide synthase
EOD	explosive ordnance disposal
EP	erythrocyte protoporphyrin
EPA	U.S. Environmental Protection Agency
EPMA	electron probe microanalysis
EPSP	excitatory postsynaptic potential
EqP	equilibrium partitioning (theory)
ERG	electroretinogram
ERL	effects range – low
ERM	effects range – median
EROD	ethoxyresorufin-*O*-deethylase
ESP	electrostatic precipitator
ESRD	end-stage renal disease
ET	endothelein; essential tremor
ET-AAS	electrothermal atomic absorption spectroscopy
EXAFS	extended X-ray absorption fine structure
EXANES	extended X-ray absorption near edge spectroscopy
F344	Fischer 344 (rat)
FA	fatty acid
FCS	fetal calf serum
FDA	Food and Drug Administration
FEF	forced expiratory flow
FEV_1	forced expiratory volume in one second
FGF	fibroblast growth factor (e.g., βFGF, αFGF)
FI	fixed-interval (operant conditioning)
FMLP	*N*-formyl-L-methionyl-L-leucyl-L-phenylalanine
fMRI	functional magnetic resonance imaging
f_{OC}	fraction organic carbon
FPLC	fast protein liquid chromatography
FR	Federal Register; fixed-ratio operant conditioning

FSH	follicle stimulating hormone
FT3	free triiodothyronine
FT4	free thyroxine
FVC	forced vital capacity
γ-GT	γ-glutamyl transferase
GABA	gamma aminobutyric acid
GAG	glycosaminoglycan
GCI	General Cognitive Index
GD	gestational day
GDP	guanosine diphosphate
GEE	generalized estimating equations
GFAAS	graphite furnace atomic absorption spectroscopy
GFAP	glial fibrillary acidic protein
GFR	glomerular filtration rate
GH	growth hormone
GI	gastrointestinal
GL	gestation and lactation
GLU	glutamate
GM	geometric mean
GMP	guanosine monophosphate
GnRH	gonadotropin releasing hormone
g_{OC}	grams organic carbon
G6PD	glucose-6-phosphate dehydrogenase
GPEI	glutathione *S*-transferase P enhancer element
G-R	Graham-Rosenblith Behavioral Examination for Newborns
GRP78	glucose-regulated protein 78
GSD	geometric standard deviation
GSD_i	individual geometric standard deviation
GSH	glutathione; reduced glutathione
GSHPx	glutathione peroxidase
GSSG	oxidized glutathione
GST	glutathione transferase; glutathione *S*-transferase
GTP	guanosine triphosphate
GvH	graft versus host (reaction)

H^+	acidity
HAP	hazardous air pollutant
Hb	hemoglobin
HBEF	Hubbard Brook Experimental Forest
H_2CO_3	carbonic acid
Hct	hematocrit
HDL	high-density lipoprotein (cholesterol)
HFE	hemochromatosis gene
HFF	human foreskin fibroblasts
HH	hydroxylamine hydrochloride
HHANES	Hispanic Health and Nutrition Examination Survey
HHC	hereditary hemochromatosis
HLA	human leukocyte antigen
HNO_3	nitric acid
H_2O_2	hydrogen peroxide
HOCl	hypochlorous acid
HOME	Home Observation for Measurement of Environment
HOS-TE-85	human osteosarcoma cells
HPG	hypothalamic-pituitary-gonadal (axis)
HPLC	high-pressure liquid chromatography
H_3PO_4	phosphoric acid
HPRT	hypoxanthine guanine phosphoribosyl transferase
HSAB	Hard-Soft Acid-Base (model)
H_2SO_4	sulfuric acid
HSPG	heparan sulfate proteoglycan
HTN	hypertension
HUD	U.S. Department of Housing and Urban Development
HY-SPLIT	hybrid single-particle Lagrangian integrated trajectory (model)
IARC	International Agency for Research on Cancer
IBL	integrated blood lead index
ICD	International Classification of Diseases
ICP	inductively coupled plasma
ICP-AES	inductively coupled plasma atomic emission spectroscopy
ICP-MS	inductively coupled plasma mass spectrometry

ICRP	International Commission on Radiological Protection
IDMS	isotope dilution mass spectrometry
IEC	intestinal epithelial cells
IEUBK	Integrated Exposure Uptake Biokinetic (model)
IFN	interferon (e.g., IFN-γ)
Ig	immunoglobulin (e.g., IgA, IgE, IgG, IgM)
IGF_1	insulin-like growth factor 1
IL	interleukin (e.g., IL-1, IL-1β, IL-4, IL-6, IL-12)
IMPROVE	Interagency Monitoring of Protected Visual Environments (network)
iNOS	inducible nitric oxide synthase
i.p., IP	intraperitoneal
IQ	intelligence quotient
IRT	interresponse time
ISCST	Industrial Source Complex Short Term (model)
IT	intrathecal
i.v., IV	intravenous
KABC	Kaufman Assessment Battery for Children
KID	Kent Infant Development Scale
KLH	keyhole limpet hemocyanin
K-pNPPase	potassium-stimulated p-nitrophenylphosphatase
KTEA	Kaufman Test of Educational Achievement
K-XRF	K-shell X-ray fluorescence
L	lactation
LAA ICP-MS	laser ablation inductively coupled plasma mass spectrometry
LC_{50}	lethal concentration (at which 50% of exposed animals die)
LDH	lactate dehydrogenase
LDL	low-density lipoprotein (cholesterol)
L-dopa	3,4-dihydroxyphenylalanine (precursor of dopamine)
LE	Long Evans (rat)
LH	luteinizing hormone
LISREL	linear structural relationships (model)
LMW	low molecular weight
LNAME, L-NAME	L-N^G-nitroarginine methyl ester

LOAEL	lowest-observed adverse effect level
LOWESS	locally weighted scatter plot smoother
LPO	lipid peroxide; lipid peroxidation
LPS	lipopolysaccharide
LT_{50}	time to reach 50% mortality
LTD	long-term depression
LTP	long-term potentiation
LVH	left ventricular hypertrophy
μPIXE	microfocused particle induced X-ray emission
MAO	monoaminoxidase
MAPK	mitogen-activated protein kinase
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MDA	malondialdehyde
MDA-TBA	malondialdehyde-thiobarbituric acid
MDI	Mental Development Index
MDRD	Modification of Diet in Renal Disease (study)
meso-DMSA	*m*-2,3-dimercaptosuccinic acid
Mg-ATPase	magnesium-dependent adenosine triphosphatase
MHC	major histocompatibility complex
MK-801	NMDA receptor antagonist
MLR	mixed lymphocyte response
MMAD	mass median aerodynamic diameters
MMSE	Mini-Mental State Examination
MN	micronuclei formation
Mn-SOD	manganese-dependent superoxide dismutase
MRFIT	Multiple Risk Factor Intervention Trial
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MRS	magnetic resonance spectroscopy
MSV	Moloney sarcoma virus
MT	metallothionein
MVV	maximum voluntary ventilation

N, n	number of observations
NA, N/A	not available
NAA	*N*-acetylaspartate; neutron activation analysis
NAAQS	National Ambient Air Quality Standards
NAC	*N*-acetyl cysteine; nucleus accumbens
NAD	nicotinamide adenine nucleotide
NADH	reduced nicotinamide adenine dinucleotide; nicotinamide adenine dinucleotide dehydrogenase
NADP	nicotinamide adenine dinucleotide phosphate
NAD(P)H, NADPH	reduced nicotinamide adenine dinucleotide phosphate
NADS	nicotinamide adenine dinucleotide synthase
NAG	*N*-acetyl-β-D-glucosaminidase
Na-K-ATPase	sodium-potassium-dependent adenosine triphosphatase
NART	North American Reading Test
NAS	Normative Aging Study
NASCAR	National Association for Stock Car Automobile Racing
NAT	*N*-acetyltransferases
NAWQA	National Water-Quality Assessment
NBAS	Brazelton Neonatal Behavioral Assessment Scale
NCEA-RTP	National Center for Experimental Assessment Division in Research Triangle Park, NC
ND	non-detectable; not detected; not determined; not done
NE	norepinephrine
NEI	National Emissions Inventory
NEPSY	Developmental Neuropsychological Assessment
NES	Neurobehavioral Evaluation System
NF-κB	nuclear transcription factor-κB
NHANES	National Health and Nutrition Examination Survey
NHEXAS	National Human Exposure Assessment Survey
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute for Standards and Technology
NK	natural killer
NMDA	*N*-methyl-D-aspartate
NMDAR	*N*-methyl-D-aspartate receptor

NO	nitric oxide
NO_2	nitrogen dioxide
NO_3	nitrate
NOD	autoimmune diabetes prone strain of mice
NOEC	no-observed-effect concentration
NOM	natural organic matter
NOS	nitric oxide synthase; not otherwise specified
NO_x	nitrogen oxide metabolites
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NTP	National Toxicology Program
NTR	neurotrophin receptor
O_2^-	superoxide ion
OAQPS	Office of Air Quality Planning and Standards
OAR	Office of Air and Radiation
OC	organic carbon
OH	hydroxyl
1,25-OH-D, 1,25-OH D_3	1,25-dihydroxyvitamin D
25-OH-D, 25-OH D_3	25-hydroxyvitamin D
O horizon	forest floor
$ONOO^-$	peroxynitrate ion
OR	odds ratio
ORD	Office of Research and Development
OS	oxidative stress
OSHA	Occupational Safety and Health Administration
p	probability value
P_{10}	probability for the occurrence of a blood lead concentration exceeding 10 µg/dL
PAD	peripheral arterial disease
PAH	polycyclic aromatic hydrocarbon
PAI-1	plasminogen activator inhibitor-1
Pb	lead
^{203}Pb	lead-203 radionuclide

^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb	stable isotopes of lead-204, -206, -207, -208 respectively
^{210}Pb	lead-210 radionuclide
PbB	blood lead; blood lead concentration
PbBPs	lead binding proteins
$PbCl_2$	lead choride
$PbCO_3$	lead carbonate
PbD	interior dust lead concentration
PBG-S	porphobilinogen synthase
PbH	hand lead concentration
$Pb(NO_3)_2$	lead nitrate
PbO	lead oxide
$Pb(OH)_2$	lead hydroxide
PbS	galena
$PbSO_4$	lead sulfate
PC12	pheochromocytoma cell
PCV	packed cell volume
PDE	phosphodiesterase
PDI	Psychomotor Index
PEC	probably effect concentration
PFCs	plaque forming cells
PG	prostaglandin (e.g., PGE_2 ,PGF_2)
PHA	phytohemagglutinin A
P_i	inorganic phosphorus
PIXE	particle induced X-ray emission
PKA	protein kinase A
PKC	protein kinase C
PM	particulate matter
PM_{10}	combination of coarse and fine particulate matter
$PM_{2.5}$	fine particulate matter
PMN	polymorphonuclear leukocyte
PMNL	polymorphonuclear leukocyte
P5N	pyrimidine 5'-nucleotidase
PND	postnatal day
POMS	Profile of Mood States

ppb	parts per billion
PPD	purified protein derivative
ppm	parts per million
PPVT-R	Peabody Picture Vocabulary Test-Revised
PRL	prolactin
PTH	parathyroid hormone
PTHrP	parathyroid hormone-related protein
PUFA	polyunsaturated fatty acid
PVC	polyvinyl chloride
PWM	pokeweed mitogen
R^2	multiple correlation coefficient
r	Pearson correlation coefficient
r^2	correlation coefficient
RAAS	renin-angiotensin-aldosterone system
RAS	renin-angiotensin system
RBA	relative bioavailablity
RBC	red blood cell; erythrocyte
RBP	retinol binding protein
^{222}Rn	most stable isotope of radon
ROI	reactive oxygen intermediate
ROS	reactive oxygen species
ROS 17.2.8	rat osteosarcoma cell line
RR	relative risk
∑SEM	sum of the molar concentrations of simultaneously extracted metal
SAB	Science Advisory Board
S-B IQ	Standford-Binet Intelligence Quotient
SBIS-4	Stanford-Binet Intelligence Scale-4th Edition
s.c., SC	subcutaneous
SCAN	test of central auditory processing
SCE	sister chromatid exchange
SD	standard deviation; Spraque-Dawley (rat)
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SE	standard error; *Staphylococcus aureus* enterotoxin

SEM	simultaneously extracted metal; standard error of the mean
SES	socioeconomic status
sGC	soluble guanylate cyclase
SHBG	sex hormone binding globulin
SIMS	secondary ion mass spectrometry
SIR	standardized incidence ratio
SLE	systemic lupus erythmatosus
SMR	standardized mortality ratio
SNAP	Schneider Neonatal Assessment for Primates
SO_2	sulfur dioxide
SOD	superoxide dismutase
SOILCHEM	chemical species equilibrium model
SRA	Self Reported Antisocial Behavior scale
SRBC	sheep red blood cell
SRC	Syracuse Research Corporation
SRD	Self Report of Delinquent Behavior
SRE	sterol regulatory element
SRIXE	synchrotron radiation induced X-ray emission
SULT	sulfotransferases
T3	triiodothyronine
T4	thyroxine
T&E	threatened and endangered (species)
TB	tuberculosis
TBA	thiobarbituric acid
TBARS	thiobarbituric acid-reactive species
T_C	cytotoxic T lymphocyte
T cell	T lymphocyte
TEC	threshold effect concentration
TEL	tetraethyllead; triethyl lead chloride
TES	testosterone
TF	transferrin
TGF	transforming growth factor (e.g., TGF-α ,TGF-β, TGF-β1)
T_H	T-helper lymphocyte
^{232}Th	stable isotope of thorium-232

Th0	precursor T lymphocyte
Th1	T-derived lymphocyte helper 1
Th2	T-derived lymphocyte helper 2
T_{HC}	CD4,CD8-positive T lymphocytes
TIMS	thermal ionization mass spectrometry
TLC	Treatment of Lead-exposed Children (study)
T_M	T-memory lymphocyte
TML	tetramethyllead
TNF	tumor necrosis factor (e.g., TNF-α, TNF-β1)
tPA	plasminogen activator
TPALL	transfer rate from diffusible plasma to all destinations
TPBS	Total Problem Behavior Score
TPY	tons per year
TRH	thyroid releasing hormone
TRI	Toxics Release Inventory
TriAL	trialkyllead
Trk	tyrosine kinase receptor
TSH	thyroid stimulating hormone
TSP	total suspended particulates
TSS	total suspended solids
TT3	total triiodothyronine
TT4	serum total thyroxine
TTR	transthyretin
TWA	time-weighted average
TX	tromboxane (e.g., TXB_2)
^{235}U, ^{238}U	uranium-234 and -238 radionuclides
UClP	plasma-to-urine clearance
UDP	uridine diphosphate
UGT	uridine diphosphate-glucuronyl transferases
UNECE	United Nations Economic Commission for Europe
USGS	United States Geological Survey
UV	ultraviolet
VC	vital capacity
VCS	vinyl chloride stabilizer

Vd	deposition velocity
VDR	vitamin D receptor
VEP	visual-evoked potential
VI	variable-interval
VLDL	very low density lipoprotein (cholesterol)
VMI	visual motor integration
VP	plasma volume
VSMC	vascular smooth muscle cells
WHO	World Health Organization
WIC	Women, Infants, and Children (program)
WISC-III	Wechsler Intelligence Scale for Children-III
WISC-R	Wechsler Intelligence Scale for Children-Revised
WPPSI	Wechsler Preschool and Primary Scale of Intelligence
WRAT-R	Wide Range Achievement Test-Revised
w/v	weight per volume
XAS	X-ray absorption spectroscopy
XPS	X-ray photoelectron spectroscopy
X-rays	synchrotron radiation
XRD	X-ray diffraction
XRF	X-ray fluorescence
ZPP	zinc protoporphyrin

EXECUTIVE SUMMARY

E.1 INTRODUCTION

This document critically assesses the latest scientific information concerning health and welfare effects associated with the presence of various concentrations of lead (Pb) in ambient air, as pertinent to providing updated scientific bases for EPA's periodic review of the National Ambient Air Quality Standards for Lead (Pb NAAQS). As such, this document builds upon previous assessments published by the U.S. Environmental Protection Agency (EPA), including: (a) the 1977 EPA document, *Air Quality Criteria for Lead*; (b) an updated revision of that Lead Air Quality Criteria Document and an accompanying Addendum published in 1986 (1986 Lead AQCD/Addendum); and (c) an associated 1990 Supplement to that 1986 AQCD/Addendum. This document focuses on evaluation and integration of information relevant to Pb NAAQS criteria development that has become available mainly since that covered by the 1986 and 1990 criteria assessments.

E.1.1 Clean Air Act Legal Requirements

As discussed in Chapter 1 of this revised Lead AQCD, Sections 108 and 109 of the Clean Air Act (CAA) govern establishment, review, and revision of U.S. National Ambient Air Quality Standards (NAAQS):

- Section 108 directs the U.S. Environmental Protection Agency (EPA) Administrator to list ubiquitous (widespread) air pollutants that may reasonably be anticipated to endanger public health or welfare and to issue air quality criteria for them. The air quality criteria are to reflect the latest scientific information useful in indicating the kind and extent of all exposure-related effects on public health and welfare expected from the presence of the pollutant in the ambient air.
- Section 109 directs the EPA Administrator to set and periodically revise, as appropriate, two types of NAAQS: (a) primary NAAQS to protect against adverse health effects of listed criteria pollutants among sensitive population groups, with an adequate margin of safety, and (b) secondary NAAQS to protect against welfare effects (e.g., impacts on vegetation, crops, ecosystems, visibility, climate, man-made materials, etc.). Section 109 also requires peer review of the NAAQS and their underlying scientific bases by the Clean Air Scientific Advisory Committee (CASAC), a committee of independent non-EPA experts.

E.1.2 Chronology of Lead NAAQS Revisions

- In 1971, U.S. EPA promulgated national ambient air standards for several major "criteria" pollutants (see Federal Register, 1971) that did not include Pb at that time. Later, on October 5, 1978, the EPA promulgated primary and secondary NAAQS for Pb, as announced in the Federal Register (1979). The primary and the secondary NAAQSs are the same: 1.5 $\mu g/m^3$ as a calendar quarterly average (maximum arithmetic mean averaged over 90 days). The standards were based on EPA's 1977 document, *Air Quality Criteria for Lead*.

- In 1986, the EPA published a revised Lead AQCD that assessed newly available scientific information published through December 1985. That 1986 document was mainly concerned with Pb health and welfare effects, but other scientific data were also discussed to provide a better understanding of the pollutant in the environment. Thus, the Lead AQCD included chapters that discussed the atmospheric chemistry and physics of the pollutant; analytical approaches; environmental concentrations; human exposure and dosimetry; physiological, toxicological, clinical, epidemiological aspects of Pb health effects; and Pb effects on ecosystems. An Addendum to the 1986 Lead AQCD was also published concurrently.

- Later, a supplement to the 1986 Lead AQCD/Addendum was published in 1990. That 1990 Supplement evaluated still newer information emerging in the published literature concerning (a) Pb effects on blood pressure and other cardiovascular endpoints and (b) the effect of Pb exposure during pregnancy and/or during the early postnatal period on birth outcomes and/or on the neonatal physical and neuropsychological development of infants and children.

- Evaluations contained in the 1986 Lead AQCD/Addendum and 1990 Supplement provided scientific inputs to support decision making regarding periodic review and, as appropriate, revision of the Pb NAAQS, and they were drawn upon by EPA's Office of Air Quality Planning and Standards (OAQPS) in preparation of a 1990 OAQPS Lead Staff Paper. After consideration of evaluations contained in these documents, EPA chose not to propose revision of the Pb NAAQS. At that time, as part of implementing a broad 1991 U.S. EPA Strategy for Reducing Lead Exposure, the Agency focused on primarily regulatory and remedial clean up efforts to reduce Pb exposure from a variety of non-air sources and media judged to pose more extensive public health risks to U.S. populations than remaining air emissions, as well as taking other actions to reduce Pb emissions to air. By 1990, annual average ambient air Pb levels had dropped in U.S. urban areas to about 0.15-0.25 $\mu g/m^3$ due to the phasedown of leaded gasoline.

- This revised Lead AQCD, prepared by EPA's National Center for Environmental Assessment (NCEA), provides scientific bases to support Clean Air Act-mandated periodic review of the Pb NAAQS. The document assesses the latest available scientific information (published mainly through December 2005) judged to be useful in deriving criteria as scientific bases for decisions on possible revision of the current Pb NAAQS.

- A separate EPA Lead Staff Paper, prepared by OAQPS in EPA's Office of Air and Radiation (OAR), draws upon key findings/conclusions from this document and, together with other analyses, develops and presents options for consideration by the EPA Administrator in regard to review, and possible revision, of the Pb NAAQS.

E.1.3 Document Organization and Structure

Volume I of this document consists of the present Executive Summary and eight main chapters of this revised Lead AQCD. Those main chapters focus primarily on interpretative evaluation of key information, whereas more detailed descriptive summarization of pertinent studies and/or supporting analyses are provided in accompanying annexes. Volume II contains (a) the annexes for Chapters 4, 5, and 6 (which assess dosimetric, toxicologic, and epidemiologic evidence regarding Pb health effects) and (b) the annex for Chapter 7 (which assesses other information on Pb ecological effects). Topics covered in the main chapters of the present AQCD are as follows:

- This Executive Summary summarizes key findings and conclusions from Chapters 1 through 8 of this revised Lead AQCD, as they pertain to background information on Pb-related atmospheric science and air quality, human exposure aspects, dosimetric considerations, health effect issues, and environmental effect issues.
- Chapter 1 provides a general introduction, including an overview of legal requirements, the chronology of past revisions of Pb-related NAAQS, and orientation to the structure of this document.
- Chapters 2 and 3 provide background information on chemistry/physics of Pb, atmospheric transport and fate, air quality, and multimedia exposure aspects to help to place the ensuing discussions of Pb health and welfare effects into perspective.
- Chapters 4 through 6 assess dosimetry aspects, toxicologic (laboratory animal) studies, and epidemiologic (observational) studies of Pb health effects.
- Chapter 7 assesses information concerning environmental effects of Pb on terrestrial and aquatic ecosystems.
- Chapter 8 then provides an integrative synthesis of key findings and conclusions derived from the preceding chapters with regard to ambient Pb concentrations, human exposures, dosimetry, health effects of importance for primary Pb NAAQS decisions, and ecosystem effects pertinent to secondary Pb NAAQS decisions.

E.2 LEAD SOURCES, EMISSIONS, AND CONCENTRATIONS AND HUMAN MULTIMEDIA EXPOSURE PATHWAYS

Lead has been observed in measurable quantities in nearly every environmental medium all over the world. Human exposure to Pb occurs through several routes, as shown in Figure E-1.

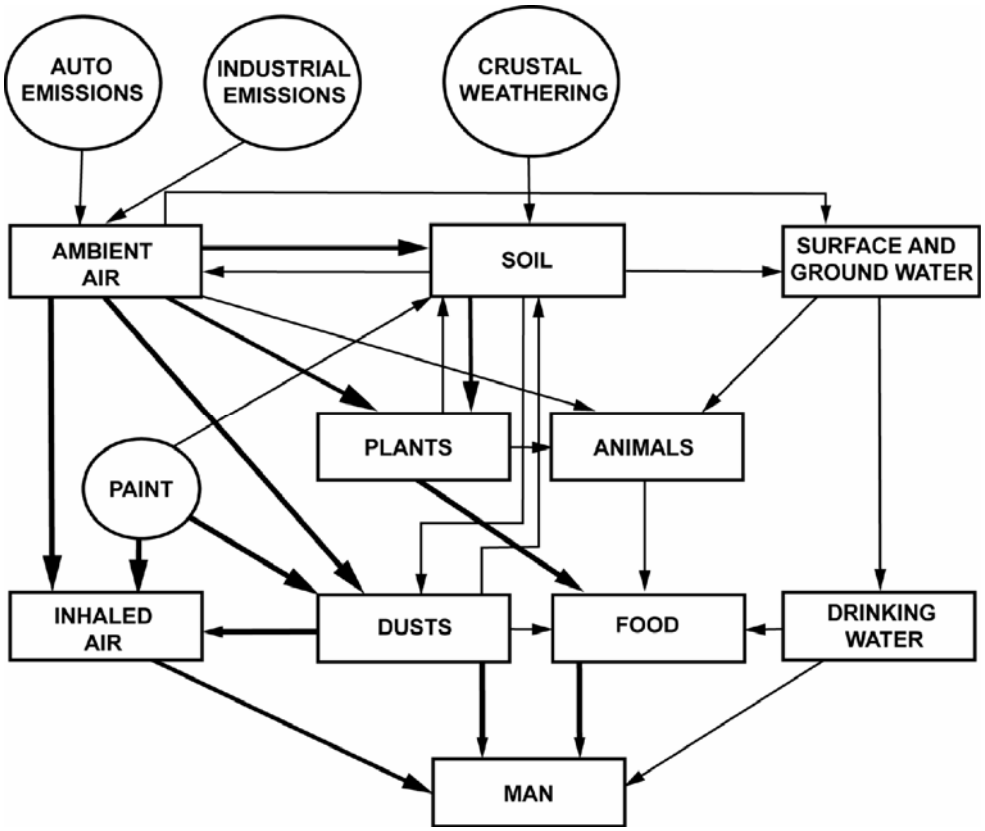

Figure E-1. Simplified diagram of environmental pathways contributing to multimedia Pb exposure of human populations.

That figure provides a simplified diagram of various routes of exposure through different environmental media, with a main focus on the ambient air. The multimedia aspects of Pb exposure can be seen in that Pb emissions to the air contribute to Pb concentrations in water, soil, and dusts; Pb in soil and dust also can make important contributions to Pb concentrations in ambient air. The relative contributions of Pb from different media and different sources on human exposure depend on factors such as the proximity of major sources to the residence and workplace of the individual, the condition of the residence (especially the presence and condition of Pb-based paint) and whether the residence is in an urban, suburban, or rural location. This section briefly summarizes available evidence concerning multimedia Pb sources and exposure pathways, with main emphasis on pathways involving airborne Pb components.

E.2.1 Ambient Lead Sources, Emissions, and Concentrations in the United States

- Overall, current U.S. ambient Pb concentrations are generally well below the Pb NAAQS level, except for locations influenced by local sources. During 2000 to 2004, on average, quarterly mean Pb concentrations at Federal Reference Method monitors ranged from 0.10 to 0.22 $\mu g/m^3$ (including point source-related monitors). In the same time period, only one to five locations from among ~200 U.S. sites measured quarterly maximum Pb levels that exceeded the NAAQS level (1.5 $\mu g/m^3$, quarterly max average) in any given year.

- Historically, mobile sources were a major source of Pb emissions, due to the use of leaded gasoline. The United States initiated the phasedown of gasoline Pb additives in the late 1970s and intensified the phase-out of Pb additives in 1990. Accordingly, airborne Pb concentrations have fallen dramatically nationwide, decreasing an average of 94% between 1983 and 2002. This is considered one of the great public and environmental health successes. Remaining mobile source-related emissions of Pb include brake wear, resuspended road dust, and emissions from vehicles that continue to use leaded gasoline (e.g., some types of aircraft and race cars).

- Currently, the major stationary sources of Pb are in the industrial sector, including iron and steel foundries; combustion sources, e.g., energy generation through coal and fuel oil combustion, or wood combustion and hazardous or solid waste incineration; primary and secondary Pb smelters; Pb-alloy production facilities; smelters for other metals, such as copper or nickel; Pb-acid battery plants; and Pb mining and/or processing.

- The resuspension of soil-bound Pb particles and contaminated road dust is a significant source of airborne Pb. In general, the main source of resuspension is wind and vehicular traffic, although resuspension through other mechanical processes such as construction, pedestrian traffic, agricultural operations, and even raindrop impaction is possible. Elevated Pb levels are found in soil near stationary Pb sources and roadways that were heavily trafficked prior to gasoline-Pb phasedown; and soil Pb can also be elevated near hazardous waste cleanup sites.

- Lead can be transported in the atmosphere through mechanisms including deposition and resuspension of Pb-containing particles. Dry deposition is the process by which pollutants are removed from the atmosphere in the absence of precipitation. The size of depositing particles is arguably the most important factor affecting dry deposition rates. Wet deposition is the process by which airborne pollutants are scavenged by precipitation and removed from the atmosphere. The size of particles can also influence wet deposition rates, with large particles being scavenged more efficiently and, hence, tending to be removed closer to their source of emission than small particles.

E.2.2 Multimedia Lead Exposure Pathways

- Exposure to Pb occurs through a number of routes. In addition to exposure to Pb in the air, other major environmental routes for exposure to Pb include: Pb in drinking water;

Pb-contaminated food; Pb in house dust; and Pb-based paint in older homes. Also, other Pb exposure sources vary in their prevalence and potential risk, such as calcium supplements, Pb-based glazes, and certain kinds of miniblinds, hair dye, and other consumer products.

- Lead-based paint exposure has long been one of the most common causes of clinical Pb toxicity. Lead-based paint was the dominant form of house paint for many decades, and a significant percentage of homes still contain Pb-based paint on some surfaces. Lead from deteriorating paint can be incorporated into exterior residential soils and/or house dust. The associated Pb exposure is often due to ingestion from hand-to-mouth activities and pica, which are common in children. Inhalation Pb exposure of adults and children can also be increased markedly during renovation or demolition projects.

- Although marked reductions of Pb in U.S. market basket food supplies have occurred during the past several decades, Pb-contaminated food still can be a major route of Pb exposure for some individuals. It was estimated that in 1990, North Americans ingested ~50 µg of Pb each day through food, beverages, and dust; with ~30 to 50% of this amount via food and beverages. With the elimination of Pb solder in U.S. canned food, food-Pb intake has fallen dramatically in the United States. Recent studies indicate that dietary Pb intake in the United States ranges from 2 to 10 µg/day. Some imported canned goods, especially from countries where Pb-soldered cans are still not banned, can be a source of notable dietary-Pb intake for some U.S. population groups, as can Pb-glazed storage pottery.

- Lead in drinking water results primarily from corrosion of Pb pipes, Pb-based solder, or brass or bronze fixtures within a residence; very little Pb in drinking water comes from utility supplies. Lead in drinking water, although generally found at low concentrations in the United States, has been linked to elevated blood Pb concentrations in some U.S. locations. In particular, the increasing use of chloramine in municipal water distribution systems, in place of chlorination as a disinfection process, has led to notable elevations of Pb in tap water in some U.S. communities in recent years.

- Given the large amount of time people spend indoors, exposure to Pb in dusts and indoor air can be significant. For children, dust ingested via hand-to-mouth activity is often a more important source of Pb exposure than inhalation. Dust can be resuspended through household activities, thereby posing an inhalation risk as well. House dust Pb can derive both from Pb-based paint and from other sources outside the home. The latter include Pb-contaminated airborne particles from currently operating industrial facilities or resuspended soil particles contaminated by deposition of airborne Pb from past emissions.

- In the United States, decreases in mobile sources of Pb, resulting from the phasedown of gasoline Pb additives, created a 98% decline in emissions from 1970 to 2003. NHANES data show a consequent parallel decline in blood-Pb levels in children aged 1 to 5 years from a geometric mean of ~15 µg/dL in 1976-1980 to ~1-2 µg/dL in the 2000-2004 period.

E.3. TOXICOKINETICS AND MEASUREMENT/MODELING OF HUMAN EXPOSURE IMPACTS ON TISSUE DISTRIBUTION OF LEAD

At the time of the 1986 Lead AQCD, it was noted that external Pb exposures via various routes (inhalation, ingestion, dermal) were reflected by increased blood-Pb concentrations, which served as a key biomarker of Pb exposure and index by which to judge risk of Pb-induced health effects. It was also recognized (a) that Pb distributed to and accumulated in several bone compartments and (b) that bone Pb might as a source of long-term internal exposure. Important findings from newly available studies include the following:

- Blood Pb is found primarily (~99%) in red blood cells. It has been suggested that the small fraction of Pb in plasma (<0.3%) may be the more biologically labile and toxicologically active fraction of the circulating Pb. The relationship between Pb intake and blood Pb concentration is curvilinear; i.e., the increment in blood Pb concentration per unit of Pb intake decreases with increasing blood Pb concentration.

- New studies investigating the kinetics of Pb in bone have demonstrated that bone Pb serves as a blood Pb source years after exposure and as a source of fetal Pb exposure during pregnancy.

- Whereas bone Pb accounts for ~70% of the body burden in children, more than 90% of the total body burden of Pb is found in the bones in human adults. Lead accumulation is thought to occur predominantly in trabecular bone during childhood and in both cortical and trabecular bone in adulthood.

- A key issue of much importance in carrying out risk assessments that estimate the potential likelihood of Pb-induced health effects is the estimation of external Pb-exposure impacts on internal Pb tissue concentrations. This includes the estimation of typical Pb-exposure impacts on internal distribution of Pb to blood and bone (as key biomarkers of Pb exposure), as well as to other "soft tissue" target organs (e.g., brain, kidney, etc.).

- Earlier criteria assessments in the 1977 and 1986 Lead AQCDs extensively discussed the available slope factor and/or other regression models of external Pb exposure impacts on blood Pb concentration in human adults and children. Further refinements in regression modeling of Pb impacts on blood or bone Pb are discussed in Chapter 4 of this document.

- The older slope factor analyses discussed in the 1977 and 1986 Lead AQCDs noted that at relatively low air-Pb concentrations (≤ 2 µg/m^3), pediatric blood-Pb levels generally increase by ~2 µg/dL per each 1 µg/m^3 increment in air-Pb concentration.

- Several new empirical analyses have shown that a child's blood Pb is strongly associated with interior dust Pb loading and its influence on hand Pb. Both exterior soil and paint Pb contribute to interior dust Pb levels. Not all ingested Pb is absorbed to the same extent. Factors such as an individual's age and diet, as well as chemical and physical properties of

Pb compounds and the media in which they occur, affect absorption, e.g., absorption is increased by fasting and dietary deficiencies in either iron or calcium. It has been estimated that for every 1000 ppm increase in soil-Pb concentration, pediatric blood-Pb levels generally increase by ~1 to 5 µg/dL in infants and children <6 years old. However, intake of soil-Pb with low bioaccessibility or bioavailability characteristics can yield distinctly lower-than-typical blood-Pb increments.

- Information on Pb biokinetics, bone mineral metabolism, and Pb exposures has led to refinements and expansions of pharmacokinetic models. Three pharmacokinetic models are currently being used or are being considered for broad application in Pb risk assessment: (1) the Integrated Exposure Uptake BioKinetic (IEUBK) model for Pb in children developed by EPA (U.S. Environmental Protection Agency, 1994a,b; White et al., 1998); (2) the Leggett model, which simulates Pb kinetics from birth through adulthood (Leggett, 1993); and (3) the O'Flaherty model, which simulates Pb kinetics from birth through adulthood (O'Flaherty, 1993, 1995).

- These models have been individually evaluated, to varying degrees, against empirical physiological data on animals and humans and data on blood Pb concentrations in individuals and/or populations (U.S. Environmental Protection Agency, 1994a,b; Leggett, 1993; O'Flaherty, 1993). In evaluating models for use in risk assessment, exposure data collected at hazardous waste sites have been used as inputs to some model simulations (Bowers and Mattuck, 2001; Hogan et al., 1998). The exposure module in the IEUBK model makes this type of evaluation feasible.

- Exposure-biokinetics models illustrate exposure-blood-body burden relationships and provide a means for making predictions about these relationships that can be experimentally or epidemiologically tested. The EPA IEUBK model for Pb has gained widespread use for risk assessment purposes in the United States and is currently clearly the model of choice in evaluating multimedia Pb exposure impacts on blood Pb levels and distribution of Pb to bone and other tissues in young children <7 years old.

- The EPA All Ages Lead Model (AALM), now under development, aims to extend beyond IEUBK capabilities to model external Pb exposure impacts (including over many years) on internal Pb distribution not only in young children, but also in older children, adolescents, young adults, and other adults well into older years. The AALM essentially uses adaptations of IEUBK exposure module features, coupled with adaptations of IEUBK biokinetics components (for young children) and of Leggett model biokinetics components (for older children and adults). However, the AALM has not yet undergone sufficient development and validation for its use yet beyond research and validation purposes.

E.4 HEALTH EFFECTS ASSOCIATED WITH LEAD EXPOSURE

Both epidemiologic and toxicologic studies have shown that environmentally relevant levels of Pb affect many different organ systems. Research completed since the 1986

AQCD/Addendum and 1990 Supplement indicates that Pb effects occur at blood-Pb even lower than those previously reported for many endpoints. Remarkable progress has been made since the mid-1980s in understanding the Pb effects on health. Recent studies have focused on details of the associations, including the shapes of concentration-response relationships, especially at levels well within the range of general population exposures, and on those biological and/or socioenvironmental factors that either increase or decrease an individual's risk. Key findings and conclusions regarding important outcomes of newly available toxicologic and epidemiologic studies of Pb health effects are highlighted below.

Neurotoxic Effects of Pb Exposure

- Neurobehavioral effects of Pb-exposure early in development (during fetal, neonatal, and later postnatal periods) in young infants and children (≤7 years old) have been observed with remarkable consistency across numerous studies involving varying study designs, different developmental assessment protocols, and diverse populations. Negative Pb impacts on neurocognitive ability and other neurobehavioral outcomes are robust in most recent studies even after adjustment for numerous potentially confounding factors (including quality of care giving, parental intelligence, and socioeconomic status). These effects generally appear to persist into adolescence and young adulthood.

- The overall weight of the available evidence provides clear substantiation of neurocognitive decrements being associated in young children with blood-Pb concentrations in the range of 5-10 µg/dL, and possibly somewhat lower. Some newly available analyses appear to show Pb effects on the intellectual attainment of preschool and school age children at population mean concurrent blood-Pb levels ranging down to as low as 2 to 8 µg/dL. A decline of 6.2 points in full scale IQ for an increase in concurrent blood Pb levels from 1 to 10 µg/dL has been estimated, based on a pooled analysis of results derived from seven well-conducted prospective epidemiologic studies.

- In the limited literature examining the effects of environmental Pb exposure on adults, mixed evidence exists regarding associations between Pb and neurocognitive performance. No associations were observed between cognitive performance and blood Pb levels; however, significant associations were observed in relation to bone Pb concentrations, suggesting that long-term cumulative Pb exposure may contribute to neurocognitive deficits in adults.

- Animal toxicology data indicate that developmental Pb exposures creating steady-state blood-Pb concentrations of ~10 µg/dL result in behavioral impairments that persist into adulthood in rats and monkeys. No evident threshold has yet been found; and Pb-induced deficits, for the most part, have been found to be very persistent, even with various chelation treatments. However, experimental studies indicate that environmental enrichment during development can partially mitigate the effects of Pb on cognitive function. In rats, neurobehavioral deficits that persisted well into adulthood were observed with prenatal,

preweaning, and postweaning Pb exposure. In monkeys, such neurobehavioral deficits were observed both with in utero-only exposure and with early postnatal-only exposure when peak blood-Pb levels did not exceed 15 µg/dL and steady-state levels were ~11 µg/dL.

- Learning impairment has been observed in animal studies at blood levels as low as 10 µg/dL, with higher level learning showing greater impairment than simple learning tasks. The mechanisms associated with these deficits include: response perseveration; insensitivity to changes in reinforcement density or contingencies; deficits in attention; reduced ability to inhibit inappropriate responding; impulsivity; and distractibility.

- Lead affects reactivity to the environment and social behavior in both rodents and nonhuman primates at blood Pb levels of 15 to 40 µg/dL. Rodent studies also show that Pb exposure potentiates the effects of stress in females.

- Auditory function has also been shown to be impaired at blood Pb levels of 33 µg/dL, while visual functions are affected at 19 µg/dL.

- Neurotoxicological studies in animals clearly demonstrated that Pb mimics calcium and affects neurotransmission and synaptic plasticity.

- Epidemiologic studies have identified genetic polymorphisms of two genes that may alter susceptibility to the neurodevelopmental consequences of Pb exposure in children. Variant alleles of the ALAD gene are associated with differences in absorption, retention, and toxicokinetics of Pb. Polymorphisms of the vitamin D receptor gene have been shown to affect the rate of resorption and excretion of Pb over time. These studies are only suggestive, and parallel animal studies have not been completed.

Cardiovascular Effects of Lead

- Epidemiologic studies have consistently demonstrated associations between Pb exposure and enhanced risk of deleterious cardiovascular outcomes, including increased blood pressure and incidence of hypertension. A meta-analysis of numerous studies estimates that a doubling of blood-Pb level (e.g., from 5 to 10 µg/dL) is associated with ~1.0 mm Hg increase in systolic blood pressure and ~0.6 mm Hg increase in diastolic pressure. Studies have also found that cumulative past Pb exposure (e.g., bone Pb) may be as important, if not more, than present Pb exposure in assessing cardiovascular effects. The evidence for an association of Pb with cardiovascular morbidity and mortality is limited but supportive.

- Experimental toxicology studies have confirmed Pb effects on cardiovascular functions. Most have shown that exposures creating blood-Pb levels of ~20 to 30 µg/dL for long periods result in arterial hypertension that persists long after cessation of Pb exposure in genetically normal animals. One study reported blood pressure increases at blood-Pb levels as low as 2 µg/dL in rats. A number of in vivo and in vitro studies provide compelling evidence for the role of oxidative stress in the pathogenesis of Pb-induced hypertension. However, experimental investigations of cardiovascular effects of Pb in animals are unclear as to why low, but not high, levels of Pb exposure cause hypertension in experimental animals.

Renal Effects of Lead

- In the general population, both circulating and cumulative Pb was found to be associated with longitudinal decline in renal function. Effects on creatine clearance have been reported in human adult hypertensives to be associated with general population mean blood-Pb levels of only 4.2 µg/dL. The public health significance of such effects is not clear, however, in view of more serious signs of kidney dysfunction being seen in occupationally exposed workers only at much higher blood-Pb levels (>30-40 µg/dL).

- Experimental studies using laboratory animals demonstrated that the initial accumulation of absorbed Pb occurs primarily in the kidneys. This takes place mainly through glomerular filtration and subsequent reabsorption, and, to a small extent, through direct absorption from the blood. Both low dose Pb-treated animals and high dose Pb-treated animals showed a "hyperfiltration" phenomenon during the first 3 months of Pb exposure. Investigations into biochemical alterations in Pb-induced renal toxicity suggested a role for oxidative stress and involvement of NO, with a significant increase in nitrotyrosine and substantial fall in urinary excretion of NO_x.

- Iron deficiency increases intestinal absorption of Pb and the Pb content of soft tissues and bone. Aluminum decreases kidney Pb content and serum creatinine in Pb-intoxicated animals. Age also has an effect on Pb retention. There is higher Pb retention at a very young age and lower bone and kidney Pb at old age, attributed in part to increased bone resorption and decreased bone accretion and, also, kidney Pb.

Effects of Lead on the Immune System

- Findings from recent epidemiologic studies suggest that Pb exposure may be associated with effects on cellular and humoral immunity. These include changes in serum immunoglobulin levels. Studies of biomarkers of humoral immunity in children have consistently found significant associations between increasing blood-Pb concentrations and serum IgE levels at blood-Pb levels <10 µg/dL.

- Toxicologic studies have shown that Pb targets immune cells, causing suppression of delayed type hypersensitivity response, elevation of IgE, and modulation of macrophages into a hyper-inflammatory phenotype. These types of changes can cause increased risk of atopy, asthma, and some forms of autoimmunity and reduced resistance to some infectious diseases. Lead exposure of embryos resulting in blood-Pb levels <10 µg/dL can produce persistent later-life immunotoxicity.

Effects of Lead on Heme Synthesis

- Lead exposure has been associated with disruption of heme synthesis in both children and adults. A 10% probability of anemia (hematocrit <35%) is estimated to be associated with a blood-Pb level of ~20 µg/dL at age 1 year. Increases in blood Pb concentration of about 20-30 µg/dL are sufficient to halve erythrocyte ALAD activity and sufficiently inhibit ferrochelatase to double erythrocyte protoporphyrin levels.

- Toxicological studies demonstrated that Pb intoxication interferes with red blood cell (RBC) survival and alters RBC mobility. Hematological parameters, such as mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, are also significantly decreased upon exposure to Pb. These effects are due to internalization of Pb by RBC. The transport of Pb across the RBC membrane is energy-independent and carrier-mediated; and the uptake of Pb appears to be mediated by an anion exchanger through a vanadate-sensitive pathway.

- Erythrocyte ALAD activity ratio (ratio of activated/non activated enzyme activity) has been shown to be a sensitive, dose-responsive measure of Pb exposure, regardless of the mode of administration of Pb. Competitive enzyme kinetic analyses in RBCs from both humans and Cynomolgus monkeys indicated similar inhibition profiles by Pb.

Effects of Lead on Bones and Teeth

- Experimental studies in animals demonstrate that Pb substitutes for calcium and is readily taken up and stored in the bone and teeth of animals, potentially allowing bone cell function to be compromised both directly and indirectly by exposure.

- Relatively short term exposure of mature animals to Pb does not result in significant growth suppression. However, chronic Pb exposure during times of inadequate nutrition has been shown to adversely influence bone growth, including decreased bone density, decreased trabecular bone, and growth plates.

- Exposure of developing animals to Pb during gestation and the immediate postnatal period has clearly been shown to significantly depress early bone growth in a dose-dependent fashion, though this effect is not manifest below a certain threshold.

- Systemically, Pb has been shown to disrupt mineralization of bone during growth, to alter calcium binding proteins, and to increase calcium and phosphorus concentration in the blood stream, in addition to potentially altering bone cell differentiation and function by altering plasma levels of growth hormone and calciotropic hormones such as vitamin D_3 [1,25-$(OH_2)D_3$.

- Periods of extensive bone remodeling, such as occur during weight loss, advanced age, altered metabolic state, and pregnancy and lactation are all associated with mobilization of Pb stores from bone of animals.

- Numerous epidemiologic studies and, separately, animal studies (both post-eruptive Pb exposure and pre- and perinatal Pb exposure studies) suggest that Pb is a caries-promoting element. However, whether Pb incorporation into the enamel surface compromises the integrity and resistance of the surface to dissolution, and ultimately increases risk of dental decay, is unclear.

- Increased risk of dental caries has been associated with Pb exposure in children and adults. Lead effects on caries were observed in populations whose mean blood-Pb levels were less than 10 μg/dL.

Reproductive and Developmental Effects of Lead

- Epidemiologic evidence suggests small associations between Pb exposure and male reproductive outcomes, including perturbed semen quality and increased time to pregnancy. There are no adequate epidemiologic data to evaluate associations between Pb exposure and female fertility. Most studies have yielded no associations, or weak associations, of Pb exposure with thyroid hormone status and male reproductive endocrine status in highly exposed occupational populations.

- New toxicologic studies support earlier conclusions, presented in the 1986 Lead AQCD, that (a) Pb can produce both temporary and persisting effects on male and female reproductive function and development and (b) Pb disrupts endocrine function at multiple points along the hypothalamic-pituitary-gonadal axis. Although there is evidence for a common mode of action, consistent effects on circulating testosterone levels are not always observed in Pb-exposed animals. Inconsistencies in reports of circulating testosterone levels complicate derivation of a dose-response relationship for this endpoint.

- Lead-induced testicular damage (ultrastructural changes in testes of monkeys at blood-Pb >35 to 40 µg/dL) and altered female sex hormone release, imprinting during early development, and altered female fertility all suggest Pb-induced reproductive effects. However, Pb exposure does not generally produce total sterility. Pre- and postnatal exposure to Pb has been demonstrated to result in fetal mortality and produce a variety of sublethal effects in the offspring. Many of the Pb-induced sublethal developmental effects occur at maternal blood-Pb levels that do not result in clinical (overt) toxicity in the mothers. Teratogenic effects resulting from Pb exposure reported in a few studies appear to be confounded by maternal toxicity.

Lead Effects on Other Organ Systems

- Lead impacts the hypothalamic-pituitary-adrenal axis, elevating corticosterone levels and altering stress responsivity. This may be a potential mechanism contributing to Pb-induced hypertension, with further possible roles in the etiology of diabetes, obesity and other disorders.

- Studies of hepatic enzyme levels in serum suggest that liver injury may be present in Pb workers; however, associations specifically with Pb exposures are not evident. Children exposed to relatively high levels of Pb (blood Pb >30 µg/dL) exhibit depressed levels of circulating 1,25-dihydroxy vitamin D (1,25-OH-D). However, associations between serum vitamin D status and blood Pb were not evident in a study of calcium-replete children who had average lifetime blood-Pb concentrations <25 µg/dL.

- Field studies that evaluated hepatic enzyme levels in serum suggest that liver injury may be present in Pb workers; however, associations specifically with Pb exposures have not been well established.

- Simultaneous induction of the activities of phase II drug metabolizing enzymes and decreased phase I enzymes with a single exposure to Pb nitrate in rat liver suggest that Pb is capable of causing biochemical phenotype similar to hepatic nodules.

- Newer studies examined the induction of GST-P at both transcriptional and translational levels using in vitro systems and indicated a role for Pb-nitrate and Pb-acetate in the induction process.

- Lead-induced alterations in cholesterol metabolism appear to be mediated by the induction of several enzymes related to cholesterol metabolism and the decrease of 7 α-hydroxylase, a cholesterol catabolizing enzyme. This regulation of cholesterol homeostasis is modulated by changes in cytokine expression and related signaling.

- Newer experimental evidence suggests that Pb-induced alterations in liver heme metabolism involve perturbations in ALAD activity, porphyrin metabolism, alterations in Transferrin gene expression, and associated changes in iron metabolism.

- Gastrointestinal (GI) absorption of Pb is influenced by a variety of factors, including chemical and physical forms of the element in ingested media, age at intake, and various nutritional factors. The degeneration of intestinal mucosal epithelium leading to potential malabsorption and alterations in the jejunal ultrastructure (possibly associated with distortion of glycocalyx layer) have been reported in the intestine of Pb-exposed rats.

- Nutritional studies that varied Pb, Ca, and vitamin D levels in the diet have demonstrated competition of Pb with Ca absorption. Supplementation with vitamin D has been reported to enhance intestinal absorption of Ca and Pb. Physiological amounts of vitamin D, when administered to vitamin D-deficient rats, resulted in elevated Pb and Ca levels. In the case of severe Ca deficiency, Pb ingestion results in a marked decrease in serum 1,25 hydroxy vitamin D.

Genotoxic and Carcinogenic Effects of Lead

- Epidemiologic studies of highly exposed occupational populations suggest a relationship between Pb and cancers of the lung and the stomach; however the evidence is limited by the presence of various potential confounders, including metal coexposures (e.g., to arsenic, cadmium), smoking, and dietary habits. The 2003 NTP and 2004 IARC reviews concluded that Pb and Pb compounds were probable carcinogens, based on limited evidence in humans and sufficient evidence in animals. Similarly, Pb and Pb compounds would likely be classified as likely to be carcinogenic to humans according to the new 2005 EPA Cancer Assessment Guidelines for Carcinogen Risk Assessment, based on animal data even though the human data are inadequate.

- Studies of genotoxicity consistently find associations of Pb exposure with DNA damage and micronuclei formation; however, the associations with the more established indicator of cancer risk, chromosomal aberrations, are inconsistent.

- Pb is an animal carcinogen and extends our understanding of mechanisms involved to include a role for metallothionein. Specifically, the recent data show that metallothionein may participate in Pb inclusion bodies and, thus, serves to prevent or reduce Pb-induced tumorigenesis.

- In vitro cell culture studies that evaluated the potential for Pb to transform rodent cells are inconsistent, and careful study of a time course of exposure is necessary to determine whether Pb actually induces transformation in cultured rodent cells. There is increased evidence suggesting that Pb may be co-carcinogenic or promotes the carcinogenicity of other compounds. Cell culture studies do support a possible epigenetic mechanism or co-mutagenic effects.

Lead-Binding Proteins

- Proteins depending upon sulfur-containing side chains for maintaining conformity or activity are vulnerable to inactivation by Pb, due to its strong sulfur-binding affinity.

- The enzyme, ALAD, a 280 kDa protein, is inducible and is the major Pb-binding protein within the erythrocyte.

- The Pb-binding protein in rat kidney has been identified as a cleavage product of α-2 microglobulin. The low molecular weight Pb-binding proteins in human kidney have been identified as thymosin β4 (molecular weight 5 kDa) and acyl-CoA binding protein (molecular weight 9 kDa). In human brain, Pb-binding proteins include thymosin β4 and an unidentified protein of 23 kDa.

- Animal toxicology studies with metallothionein-null mice demonstrated a possible role for metallothionein as a renal Pb-binding protein.

E.5 HUMAN POPULATION GROUPS AT SPECIAL RISK AND POTENTIAL PUBLIC HEALTH IMPACTS

- Children, in general and especially low SES (often including larger proportions of African-American and Hispanic) children, have been well-documented as being at increased risk for Pb exposure and Pb-induced adverse health effects. This is due to several factors, including enhanced exposure to Pb via ingestion of soil-Pb and/or dust-Pb due to normal hand-to-mouth activity and/or pica.

- Even children with low Pb exposure levels (having blood Pb of 5-10 μg/dL or, possibly, somewhat lower) are at notable risk, due to apparent non-linear dose-response relationships between blood Pb and neurodevelopmental outcomes. It is hypothesized that initial neurodevelopmental lesions occurring at blood-Pb levels <10 μg/dL may disrupt different developmental processes in the nervous system than more severe high level exposures.

- Adults with idiosyncratic exposures to Pb through occupations, hobbies, make-up use, glazed pottery, native medicines, and other sources are at risk for Pb toxicity. Certain ethnic and racial groups are known to have cultural practices that involve ingestion of Pb-containing substances, e.g., ingestion of foods or beverages stored in Pb-glazed pottery or imported canned food from countries that allow Pb-soldered cans.

- Cumulative past Pb exposure, measured by bone Pb, may be a better predictor of cardiovascular effects than current blood-Pb levels. African-Americans are known to have substantially higher baseline blood pressure than other ethnic groups, so Pb's impact on an already higher baseline could indicate a greater susceptibility to Pb for this group.

- Effects on adults of low-level Pb exposures also include some renal effects (i.e., altered creatinine clearance) at blood-Pb levels <5 ug/dL. Lead exposure combined with other risk factors, such as diabetes, hypertension, or chronic renal insufficiency may result in clinically-relevant effects in individuals with two or more other risk factors.

- At least two genetic polymorphisms, of the ALAD and the vitamin D receptor gene, have been suggested to play a role in susceptibility to Pb. In one study, African-American children were found to have a higher incidence of being homozygous for alleles of the vitamin D receptor gene thought to contribute to greater Pb blood levels. This work is preliminary and further studies will be necessary to determine implications of genetic differences that may make certain populations more susceptible to Pb exposure.

- What was considered "low" for Pb exposure levels in the 1980s is an order of magnitude higher than the current mean level in the U.S. population, and current average blood-Pb levels in U.S. populations remain perhaps as much as two orders of magnitude above pre-industrial "natural" levels in humans. There is no level of Pb exposure that has yet been identified, with confidence, as being clearly not being associated with possible risk of deleterious health effects. Some recent studies of Pb neurotoxicity in infants have observed effects at population average blood-Pb levels of only 1 or 2 µg/dL; and some cardiovascular, renal, and immune outcomes have been reported at blood-Pb levels below 5 µg/dL.

- Public health interventions have resulted in declines, over the last 25 years, of more than 90% in the mean blood-Pb level within all age and gender subgroups of the U.S. population, substantially decreasing the numbers of individuals at likely risk for Pb-induced toxicities. Nevertheless, estimates of the magnitude of potential public health impacts of Pb exposure can be substantial for the U.S. population. For example, in estimating the effect of Pb exposure on intelligence, it was projected that the fraction of individuals with an IQ >120 would decrease from ~9% with no Pb exposure to less than 3% at a blood-Pb level of 10 µg/dL. Also, the fraction of individuals with an IQ >130 points was estimated as being likely to decrease from 2.25% to 0.5% with a blood-Pb level change from 0 to 10 µg/dL. In addition, an estimate of hypertension-related risk for serious cardiovascular events (coronary disease, stroke, peripheral artery disease, cardiac failure) indicates that a decrease in blood Pb from 10 to 5 µg/dL could result in an annual decrease of 27 events per 100,000 women and 39 events per 100,000 men.

E.6 ENVIRONMENTAL EFFECTS OF LEAD

Chapter 7 assesses the environmental effects of Pb, including discussion, in particular, of Pb effects on terrestrial and aquatic ecosystems and the methodological approaches used to study such effects.

E.6.1 Terrestrial Ecosystems

Methodologies Used in Terrestrial Ecosystem Research

- Electron probe microanalysis (EPMA) techniques provide the greatest information on metal speciation. Other techniques, such as EXAFS (extended X-ray absorption fine structure) and EXANES (extended X-ray absorption near edge spectroscopy), show great promise and will be important in solving key mechanistic questions.

- In situ methodologies have been developed to lower soil-Pb relative bioavailability. These amendments typically fall within the categories of phosphate, biosolid, and Al/Fe/Mn-oxide amendments. Some of the drawbacks to soil amendment include phosphate toxicity to plants and increased arsenic mobility at high soil phosphate concentrations. The use of iron(III) phosphate seems to mitigate arsenic mobility, however increased concentrations of phosphate and iron limit their application when drinking water quality is a concern.

Distribution of Atmospherically Delivered Lead in Terrestrial Ecosystems

- Total Pb deposition during the 20th century has been estimated at 1 to 3 g Pb m^{-2}, depending on elevation and proximity to urban areas. Total contemporary loadings to terrestrial ecosystems are ~1 to 2 mg m^{-2} $year^{-1}$. This is a relatively small annual flux of Pb compared to the reservoir of ~0.5 to 4 g m^{-2} of gasoline additive-derived Pb already deposited in surface soils over much of the United States.

- Dry deposition can account for 10% to >90% of total Pb deposition. Because Clean Air Act Legislation has preferentially reduced Pb associated with fine particles, relative contributions of dry deposition have changed in the last few decades.

- Although inputs of Pb to ecosystems are currently low, Pb export from watersheds via groundwater and streams is substantially lower than inputs. Therefore, even at current input levels, watersheds are accumulating anthropogenic Pb.

- Species of Pb delivered to terrestrial ecosystems can be inferred by emission source. For example, Pb species emitted from automobile exhaust are dominated by particulate Pb halides and double salts with ammonium halides (e.g., PbBrCl, $PbBrCl_2NH_4Cl$), while Pb emitted from smelters is dominated by Pb-sulfur species. Halides from automobile exhaust break down rapidly in the atmosphere, via redox reactions in the presence of atmospheric acids. Lead phases in the atmosphere, and presumably the compounds delivered to the

surface of the earth (i.e., to vegetation and soils), are suspected to be in the form of $PbSO_4$, PbS, and PbO.

- The importance of humic and fulvic acids and hydrous Mn- and Fe-oxides for scavenging Pb in soils was discussed in some detail in the 1986 Lead AQCD. The importance of these Pb binding substrates is reinforced by studies reported in the more contemporary literature.

- The amount of Pb that has leached into mineral soil appears to be on the order of 20 to 50% of the total anthropogenic Pb deposition.

- The vertical distribution and mobility of atmospheric Pb in soils was poorly documented prior to 1986. Techniques using radiogenic Pb isotopes have been developed to differentiate between gasoline-derived Pb and natural, geogenic (native) Pb. These techniques provide more accurate determinations of the depth-distribution and potential migration velocities for atmospherically delivered Pb in soils.

- Selective chemical extractions have been used extensively over the past 20 years to quantify amounts of a particular metal phase in soil or sediment rather than total metal concentration. However, some problems persist with the selective extraction technique: (a) extractions are rarely specific to a single phase; and (b) in addition to the nonselectivity of reagents, significant metal redistribution has been found to occur during sequential chemical extractions. Thus, although chemical extractions provide some useful information on metal phases in soil or sediment, the results should be treated as "operationally defined," e.g., "H_2O_2-liberated Pb" rather than "organic Pb."

- Soil solution dissolved organic matter content and pH typically have very strong positive and negative correlations, respectively, with the concentration of dissolved Pb species.

Effects of Lead on Natural Terrestrial Ecosystems

- Atmospheric Pb pollution has resulted in the accumulation of Pb in terrestrial ecosystems throughout the world. In the United States, anthropogenically-derived Pb represents a significant fraction of the total Pb burden in soils, even in sites remote from smelters and other industrial plants. However, few significant effects of Pb pollution have been observed at sites that are not near point sources of Pb.

- Evidence from precipitation collection and sediment analyses indicates that atmospheric deposition of Pb has declined dramatically (>95%) at sites unaffected by point sources of Pb, and there is little evidence that Pb accumulated in soils at these sites represents a threat to ground water or surface water supplies.

- The effects of Pb and other chemical emissions on terrestrial ecosystems near smelters and other industrial sites decrease downwind from the Pb source. Several studies using the soil burden as an indicator have shown that much of the contamination occurs within a radius of 20 to 50 km around the emission source. Elevated metal concentrations around smelters have been found to persist despite significant reductions in emissions. The concentrations of Pb in soils, vegetation, and fauna at these sites can be two to three orders of magnitude

higher than in reference areas. Assessing the risks specifically associated with Pb is difficult, because these sites also experience elevated concentrations of other metals and because of effects related to SO_2 emissions. The confounding effect of other pollutants makes the assessment of Pb-specific exposure-response relationships impossible at the whole-ecosystem level.

- In the most extreme cases, near smelter sites, the death of vegetation causes a near-complete collapse of the detrital food web, creating a terrestrial ecosystem in which energy and nutrient flows are minimal.

- More commonly, stress in soil microorganisms and detritivores can cause reductions in the rate of decomposition of detrital organic matter. Although there is little evidence of significant bioaccumulation of Pb in natural terrestrial ecosystems, reductions in microbial and detritivorous populations can affect the success of their predators. Thus, at present, industrial point sources represent the greatest Pb-related threat to the maintenance of sustainable, healthy, diverse, and high-functioning terrestrial ecosystems in the United States.

Terrestrial Species Response/Mode of Action

- Plants take up Pb via their foliage and through their root systems. Surface deposition of Pb onto plants may represent a significant contribution to the total Pb in and on the plant, as has been observed for plants near smelters and along roadsides.

- There are two possible mechanisms (symplastic or apoplastic) by which Pb may enter the root of a plant. The symplastic route is through the cell membranes of root hairs; this is the mechanism of uptake for water and nutrients. The apoplastic route is an extracellular route between epidermal cells into the intercellular spaces of the root cortex. The symplastic route is considered the primary mechanism of Pb uptake in plants.

- Recent work supports previous conclusions that the form of metal tested, and its speciation in soil, influence uptake and toxicity to plants and invertebrates. The oxide form of Pb is less toxic than the chloride or acetate forms, which are less toxic that the nitrate form of Pb. However, these results must be interpreted with caution, as the counter ion (e.g., the nitrate ion) may also be contributing to the observed toxicity.

- Lead may be detoxified in plants by deposition in root cell walls, and this may be influenced by calcium concentrations. Other hypotheses put forward recently include the presence of sulfur ligands and the sequestration of Pb in old leaves as detoxification mechanisms. Lead detoxification has not been studied extensively in invertebrates. Glutathione detoxification enzymes were measured in two species of spider. Lead may be stored in waste nodules in earthworms or as pyromorphite in the nematode.

- Lead effects on heme synthesis (as measured primarily by ALAD activity and protoporphyrin concentration) were documented in the 1986 Lead AQCD and continue to be studied. However, researchers caution that changes in ALAD and other enzyme parameters are not always related to adverse effects, but may simply indicate exposure. Other effects on plasma enzymes, which may damage other organs, have been reported. Lead also may cause lipid

peroxidation, which may be alleviated by vitamin E, although Pb poisoning may still result. Changes in fatty acid production have been reported, which may influence immune response and bone formation.

- Insectivorous mammals may be more exposed to Pb than herbivores, and higher trophic-level consumers may be less exposed than lower trophic-level organisms. Nutritionally-deficient diets (including low calcium) cause increased uptake of Pb and greater toxicity in birds.

- Interactions of Pb with other metals are inconsistent, depending on the endpoint measured, the tissue analyzed, the animal species, and the metal combination.

Exposure/Response of Terrestrial Species

- Recent critical advancements reported in the current Lead AQCD in understanding toxicity levels relies heavily on the work completed by a multi-stakeholder group, consisting of federal, state, consulting, industry, and academic participants, led by the EPA to develop Ecological Soil Screening Levels (Eco-SSLs).

- Eco-SSLs are concentrations of contaminants in soils that would result in little or no measurable effect on ecological receptors. The Eco-SSLs are intentionally conservative in order to provide confidence that contaminants which could present an unacceptable risk are not screened out early in the evaluation process. That is, at or below these levels, adverse effects are considered unlikely. Due to conservative modeling assumptions (e.g., metal exists in most toxic form or highly bioavailable form, high food ingestion rate, high soil ingestion rate) which are common to screening processes, several Eco-SSLs are derived below the average background soil concentration for a particular contaminant.

- The Eco-SSLs for terrestrial plants, birds, mammals, and soil invertebrates are 120, 11, 56, and 1700 mg Pb/kg soil, respectively.

E.6.2 Aquatic Ecosystems

Methodologies Used in Aquatic Ecosystem Research

- Many of the terrestrial methods can also be applied to suspended solids and sediments collected from aquatic ecosystems. Just as in the terrestrial environment, the speciation of Pb and other trace metals in natural freshwaters and seawater plays a crucial role in determining their reactivity, mobility, bioavailability, and toxicity. Many of the same speciation techniques employed for the speciation of Pb in terrestrial ecosystems are applicable in aquatic ecosystems.

- There is now a better understanding of the potential effects of sampling, sample handling, and sample preparation on aqueous-phase metal speciation. Thus, a need has arisen for dynamic analytical techniques that are able to capture a metal's speciation, in-situ and in real time.

- With few exceptions, ambient water quality criteria (AWQC) are derived based on data from aquatic toxicity studies conducted in the laboratory. In general, both acute (short term) and chronic (long term) AWQCs are developed. Depending on the species, the toxicity studies considered for developing acute criteria range in length from 48 to 96 hours.

- Acceptable chronic toxicity studies should encompass the full life cycle of the test organism, although for fish, early life stage or partial life cycle toxicity studies are considered acceptable. Acceptable endpoints include reproduction, growth and development, and survival, with the effect levels expressed as the chronic value.

- The biotic ligand model (BLM), which considers the binding of free metal ion to the site of toxic action and competition between metal species and other ions, has been developed to predict the toxicity of several metals under a variety of water quality conditions. However, there are limitations to this tool in deriving AWQC because, currently, limited work has been conducted in developing chronic BLMs (for any metals, let alone Pb) and the acute BLMs to date do not account for dietary metal exposures.

Distribution of Lead in Aquatic Ecosystems

- Atmospheric Pb is delivered to aquatic ecosystems primarily through deposition (wet and/or dry) or through erosional transport of soil particles.

- A significant portion of Pb in the aquatic environment exists in the undissolved form (i.e., bound to suspended particulate matter). The ratio of Pb in suspended solids to Pb in filtrate varies from 4:1 in rural streams to 27:1 in urban streams.

- The oxidation potential of Pb is high in slightly acidic solutions, and Pb^{2+} binds with high affinity to sulfur-, oxygen-, and nitrogen-containing ligands. Therefore, speciation of Pb in the aquatic environment is controlled by many factors (e.g., pH, redox, dissolved organic carbon, sulfides). The primary form of Pb in aquatic environments is divalent (Pb^{2+}), while Pb^{4+} exists only under extreme oxidizing conditions. Labile forms of Pb (e.g., Pb^{2+}, $PbOH^{+}$, $PbCO_3$) are a significant portion of the Pb inputs to aquatic systems from atmospheric washout. Lead is typically present in acidic aquatic environments as $PbSO_4$, $PbCl_4$, ionic Pb, cationic forms of Pb-hydroxide, and ordinary Pb-hydroxide ($Pb(OH)_2$). In alkaline waters, common species of Pb include anionic forms of Pb-carbonate ($Pb(CO_3)$) and $Pb(OH)_2$.

- Lead concentrations in lakes and oceans were generally found to be much lower than those measured in the lotic waters assessed by NAWQA. In open waters of the North Atlantic the decline of Pb concentrations has been associated with the phasing out of leaded gasoline in North America and Western Europe. However, in estuarine systems, it appears that similar declines following the phase-out of leaded gasoline are not necessarily as rapid.

- Based on a synthesis of NAWQA data from the United States, Pb concentrations in surface waters, sediments, and fish tissues (whole body) respectively range from: 0.04 to 30 µg/L (mean = 0.66, median = 0.50, 95th %tile = 1.1); 0.5 to 12,000 mg/kg (mean = 120, median = 28, 95th %tile = 200); and 0.08 to 23 mg/kg (mean = 1.03, median = 0.59, 95th %tile = 3.24).

Effects of Lead on Natural Aquatic Ecosystems

- Lead exposure may adversely affect organisms at different levels of organization, i.e., individual organisms, populations, communities, or ecosystems. Generally, however, there is insufficient information available for single materials in controlled studies to permit evaluation of specific impacts on higher levels of organization (beyond the individual organism). Potential effects at the population level or higher are, of necessity, extrapolated from individual level studies. Available population, community, or ecosystem level studies are typically conducted at sites that have been contaminated or adversely affected by multiple stressors (several chemicals alone or combined with physical or biological stressors). Therefore, the best documented links between Pb and effects on the environment are with effects on individual organisms.

- Natural systems frequently contain multiple metals, making it difficult to attribute observed adverse effects to single metals. For example, macroinvertebrate communities have been widely studied with respect to metals contamination and community composition and species richness. In these studies, multiple metals were evaluated and correlations between observed community level effects were ascertained. The results often indicate a correlation between the presence of one or more metals (or total metals) and the negative effects observed. While, correlation may imply a relationship between two variables, it does not imply causation of effects.

- In simulated microcosms or natural systems, environmental exposure to Pb in water and sediment has been shown to affect energy flow and nutrient cycling and benthic community structure.

- In field studies, Pb contamination has been shown to significantly alter the aquatic environment through bioaccumulation and alterations of community structure and function.

- Exposure to Pb in laboratory studies and simulated ecosystems may alter species competitive behaviors, predator-prey interactions, and contaminant avoidance behaviors. Alteration of these interactions may have negative effects on species abundance and community structure.

- In natural aquatic ecosystems, Pb is often found coexisting with other metals and other stressors. Thus, understanding the effects of Pb in natural systems is challenging given that observed effects may be due to cumulative toxicity from multiple stressors.

Aquatic Species Response/Mode of Action

- Recent research has suggested that due to the low solubility of Pb in water, dietary Pb (i.e., Pb adsorbed to sediment, particulate matter, and food) may contribute substantially to exposure and toxicity in aquatic biota.

- Generally speaking, aquatic organisms exhibit three Pb accumulation strategies: (1) accumulation of significant Pb concentrations with a low rate of loss, (2) excretion of Pb roughly in balance with availability of metal in the environment, and (3) weak net accumulation due to very low metal uptake rate and no significant excretion.

- Protists and plants produce intracellular polypeptides that form complexes with Pb. Macrophytes and wetland plants that thrive in Pb-contaminated regions have developed translocation strategies for tolerance and detoxification.

- Like aquatic plants and protists, aquatic animals detoxify Pb by preventing it from being metabolically available, though their mechanisms for doing so vary. Invertebrates use lysosomal-vacuolar systems to sequester and process Pb within glandular cells. They also accumulate Pb as deposits on and within skeletal tissue, and some can efficiently excrete Pb. Fish scales and mucous chelate Pb in the water column, and potentially reduce visceral exposure.

- Numerous studies have reported the effects of Pb exposure on blood chemistry in aquatic biota. Plasma cholesterol, blood serum protein, albumin, and globulin concentrations were identified as bioindicators of Pb stress in fish.

- Nutrients affect Pb toxicity in aquatic organisms. Some nutrients seem capable of reducing toxicity. Exposure to Pb has not been shown to reduce nutrient uptake ability, though it has been demonstrated that Pb exposure may lead to increased production and loss of organic material (e.g., mucus and other complex organic ligands).

- Avoidance responses are actions performed to evade a perceived threat. Some aquatic organisms have been shown to be quite adept at avoiding Pb in aquatic systems, while others seem incapable of detecting its presence.

- The two most commonly reported Pb-element interactions are between Pb and calcium and between Pb and zinc. Both calcium and zinc are essential elements in organisms and the interaction of Pb with these ions can lead to adverse effects both by increased Pb uptake and by a decrease in Ca and Zn required for normal metabolic functions.

Exposure/Response of Aquatic Species

- The 1986 Lead AQCD reviewed data in the context of sublethal effects of Pb exposure. The document focused on describing the types and ranges of Pb exposures in ecosystems likely to adversely impact domestic animals. As such, the 1986 AQCD did not provide a comprehensive analysis of the effects of Pb to most aquatic primary producers, consumers, and decomposers.

- Waterborne Pb is highly toxic to aquatic organisms, with toxicity varying with the species and life stage tested, duration of exposure, form of Pb tested, and water quality characteristics.

- Among the species tested, aquatic invertebrates, such as amphipods and water fleas, were the most sensitive to the effects of Pb, with adverse effects being reported at concentrations as low as 0.45 µg/L (range: 0.45 to 8000 µg/L).

- Freshwater fish demonstrated adverse effects at concentrations ranging from 10 to >5400 µg/L, depending generally upon water quality parameters.

- Amphibians tend to be relatively Pb tolerant; however, they may exhibit decreased enzyme activity (e.g., ALAD reduction) and changes in behavior (e.g., hypoxia response behavior).

E.6.3 Critical Loads for Lead in Terrestrial and Aquatic Ecosystems

- Critical loads are defined as threshold deposition rates of air pollutants that current knowledge indicates will not cause long-term adverse effects to ecosystem structure and function. A critical load is related to an ecosystem's sensitivity to anthropogenic inputs of a specific chemical.

- The critical loads approach for sensitive ecosystems from acidification has been in use throughout Europe for about 20 years. Its application to Pb and other heavy metals in Europe is more recent. European critical load values for Pb have been developed but are highly specific to the bedrock geology, soil types, vegetation, and historical deposition trends in each European country. To date, the critical loads framework has not been used for regulatory purposes in the United States for any chemical. Considerable research is necessary before critical load estimates can be formulated for ecosystems extant in the United States.

- Speciation strongly influences the toxicity of Pb in soil and water and partitioning between dissolved and solid phases determines the concentration of Pb in soil drainage water, but it has not been taken into account in most of the critical load calculations for Pb performed to date.

- Runoff of Pb from soil may be the major source of Pb into aquatic systems. However, little attempt has been made to include this source into critical load calculations for aquatic systems due to the complexity of including this source in the critical load models.

In summary, due to the deposition of Pb from past practices (e.g., leaded gasoline, ore smelting) and the long residence time of Pb in many aquatic and terrestrial ecosystems, a legacy of environmental Pb burden exists, over which is superimposed much lower contemporary Pb loadings. The potential for ecological effects of the combined legacy and contemporary Pb burden to occur is a function of the bioavailability or bioaccessibility of the Pb, which, in turn, is highly dependent upon numerous site factors (e.g., soil organic carbon content, pH, water hardness). Moreover, while the more localized ecosystem impacts observed around smelters are often striking, these perturbations cannot be attributed solely to Pb. Many other stressors (e.g., other heavy metals, oxides of sulfur and nitrogen) can also act singly or in concert with Pb to cause such notable environmental impacts.

1. INTRODUCTION

The present document critically assesses the latest scientific information concerning health and welfare effects associated with the presence of various concentrations of lead (Pb) in ambient air, as pertinent to providing updated scientific bases for EPA's current periodic review of the National Ambient Air Quality Standards for Lead (Pb NAAQS). As such, this document builds upon previous assessments published by the U.S. Environmental Protection Agency (EPA), including: (a) the document, *Air Quality Criteria for Lead* (U.S. Environmental Protection Agency, 1977); (b) an updated revision of that Lead Air Quality Criteria Document (Lead AQCD) and an accompanying Addendum published in 1986 (U.S. Environmental Protection Agency, 1986a, b); as well as (c) an associated 1990 Supplement (U.S. Environmental Protection Agency, 1990). This document focuses on evaluation and integration of information relevant to Pb NAAQS criteria development that has become available mainly since that covered by the 1986 and 1990 criteria assessments.

This introductory chapter (Chapter 1) of the revised Lead AQCD presents: (a) background information on pertinent Clean Air Act legislative requirements, the criteria and NAAQS review process, and the history of previous Lead criteria reviews; (b) an overview of the current Lead criteria review process, associated key milestones, and schedule; and (c) an orientation to the general organizational structure and content of this revised Lead AQCD.

1.1 LEGAL AND HISTORICAL BACKGROUND

1.1.1 Legislative Requirements

Two sections of the Clean Air Act (CAA) govern the establishment, review, and revision of NAAQS. Section 108 (42 U.S.C. 7408) directs the Administrator of the U.S. Environmental Protection Agency (EPA) to identify ambient air pollutants that may be reasonably anticipated to endanger public health or welfare and to issue air quality criteria for them (U.S. Code, 2003a). These air quality criteria are to reflect the latest scientific information useful in indicating the kind and extent of all identifiable effects on public health or welfare that may be expected from the presence of a given pollutant in ambient air.

Section 109(a) of the CAA (42 U.S.C. 7409) directs the Administrator of EPA to propose and promulgate primary and secondary NAAQS for pollutants identified under Section 108 (U.S. Code, 2003b). Section 109(b)(1) defines a primary standard as one that, in the judgment of the EPA Administrator, is requisite to protect the public health (see inset below) based on the criteria and allowing for an adequate margin of safety. The secondary standard, as defined in Section 109(b)(2), must specify a level of air quality that, in the judgment of the Administrator, is requisite to protect the public welfare (see inset below) from any known or anticipated adverse effects associated with the presence of the pollutant in ambient air, based on the criteria.

EXAMPLES OF PUBLIC HEALTH EFFECTS

- Effects on the health of the general population, or identifiable groups within the population, who are exposed to pollutants in ambient air
- Effects on mortality
- Effects on morbidity
- Effects on other health conditions including indicators of:
  - pre-morbid processes,
  - risk factors, and
  - disease

EXAMPLES OF PUBLIC WELFARE EFFECTS

- Effects on personal comfort and well-being
- Effects on economic values
- Deterioration of property
- Hazards to transportation
- Effects on the environment, including:
  - animals
  - climate
  - crops
  - materials
  - soils
  - vegetation
  - visibility
  - water
  - weather
  - wildlife

Section 109(d) of the CAA (42 U.S.C. 7409) requires periodic review and, as appropriate, revision of existing criteria and standards (U.S. Code, 2003b). If, in the EPA Administrator's judgment, the Agency's review and revision of criteria make appropriate the proposal of new or revised standards, such standards are to be revised and promulgated in accordance with Section 109(b). Alternatively, the Administrator may find that revision of the standards is inappropriate and conclude the review by leaving the existing standards unchanged. Section 109(d)(2) of the 1977 CAA Amendments also requires that an independent scientific review committee be established to advise the EPA Administrator on NAAQS matters, including the scientific soundness of criteria (scientific bases) supporting NAAQS decisions. This role is fulfilled by the Clean Air Scientific Advisory Committee (CASAC), which is administratively supported by U.S. EPA's Science Advisory Board (SAB).

1.1.2 Criteria and NAAQS Review Process

Periodic reviews by EPA of criteria and NAAQS for a given criteria air pollutant progress through a number of steps, starting with the preparation of an air quality criteria document (AQCD) by the National Center for Environmental Assessment Division in Research Triangle Park, NC (NCEA-RTP), a unit within EPA's Office of Research and Development (ORD). The AQCD provides a critical assessment of the latest available scientific information upon which the NAAQS are to be based. Drawing upon the AQCD, the Office of Air Quality Planning and Standards (OAQPS), a unit within EPA's Office of Air and Radiation (OAR), prepares a Staff Paper that (a) evaluates policy implications of key studies and scientific information assessed in the AQCD; (b) presents relevant exposure and risk analyses; and (c) also presents EPA staff conclusions and recommendations for standard-setting options for the EPA Administrator to consider. The Staff Paper is intended to help "bridge the gap" between the scientific assessment contained in the AQCD and the judgments required of the Administrator in determining whether it is appropriate to retain or to revise the subject NAAQS.

Iterative drafts of both the AQCD and the Staff Paper (as well as other analyses, such as associated exposure and/or risk assessments supporting the Staff Paper) are made available for public comment and CASAC review. Final versions of the AQCD and Staff Paper incorporate changes in response to CASAC review and public comment. Based on the information in these documents, the EPA Administrator proposes decisions on whether to retain or revise the subject NAAQS, taking into account public comments and CASAC advice and recommendations. The Administrator's proposed decisions are published in the *Federal Register*, with a preamble that delineates the rationale for the decisions and solicits public comment. After considering comments received on the proposed decisions, the Administrator makes a final decision that is promulgated via a *Federal Register* notice that addresses significant comments received on the proposal.

Promulgated NAAQS decisions involve consideration of the four basic elements of a standard: *indicator*, *averaging time*, *form*, and *level*. The indicator defines the pollutant to be measured in the ambient air for the purpose of determining compliance with the standard. The averaging time defines the time period over which air quality measurements are to be obtained and averaged, considering evidence of effects associated with various time periods of exposure. The form of a standard defines the air quality statistic that is to be compared to the

level of the standard (i.e., an ambient concentration of the indicator pollutant) in determining whether an area attains the standard. The form of the standard specifies the air quality measurements that are to be used for compliance purposes (e.g., the 98th percentile of an annual distribution of daily concentrations; the annual arithmetic average), the monitors from which the measurements are to be obtained (e.g., one or more population-oriented monitors in an area), and whether the statistic is to be averaged across multiple years. These basic elements of a standard are the primary focus of the staff conclusions, and recommendations posed in the Staff Paper are explicitly specified in the ensuing NAAQS rulemaking, building upon the policy-relevant scientific information assessed in the AQCD and on policy analyses contained in the Staff Paper. These four elements, taken together, determine the degree of public health and welfare protection afforded by the NAAQS.

1.1.3 Regulatory Chronology

In 1971, U.S. EPA promulgated national ambient air standards for several major "criteria" pollutants (see Federal Register, 1971), but did not include Pb among them at that time. Later, on October 5, 1978, the EPA promulgated primary and secondary Pb NAAQS under Section 109 of the CAA (43 FR 46258), as announced in the Federal Register (1979). Identical primary and the secondary Pb standards were established at the time: 1.5 $\mu g/m^3$ as a quarterly average (maximum arithmetic mean averaged over a calendar quarter). Those standards were based on scientific assessments in EPA's original Air Quality Criteria for Lead (U.S. Environmental Protection Agency, 1977) or "1977 Lead AQCD."

In 1986, the EPA published a revised Lead AQCD (U.S. Environmental Protection Agency, 1986a). The 1986 Lead AQCD assessed newly available scientific information on the health and welfare effects associated with exposure to various concentrations of Pb in ambient air, based on literature published through 1985. That 1986 document was principally concerned with the health and welfare effects of Pb, but other scientific data were also discussed in order to provide a better understanding of the pollutant in the environment. Thus, the 1986 document included chapters that discussed the atmospheric chemistry and physics of the pollutant; analytical approaches; environmental concentrations; human exposure and dosimetry; physiological, toxicological, clinical, and epidemiological aspects of Pb health effects; and Pb effects on ecosystems. An Addendum to the 1986 Lead AQCD was also published along with it

(U.S. Environmental Protection Agency, 1986b). Subsequently, a Supplement to the 1986 Lead AQCD/Addendum was published by EPA in 1990 (U.S. Environmental Protection Agency, 1990a). That 1990 Supplement evaluated still newer information emerging in the published literature concerning (a) Pb effects on blood pressure and other cardiovascular endpoints and (b) the effects of Pb exposure during pregnancy or during the early postnatal period on birth outcomes and/or on the neonatal physical and neuropsychological development of affected infants and children.

The evaluations contained in the 1986 Lead AQCD/Addendum and 1990 Supplement provided scientific inputs to support decision-making regarding CAA-mandated periodic review and, as appropriate, revision of the Pb NAAQS; and they were drawn upon by EPA's Office of Air Quality Planning and Standards in preparation of an associated OAQPS Lead Staff Paper (U.S. Environmental Protection Agency, 1990b). Based on the scientific assessment in the 1986 Lead AQCD/Addendum and the 1990 Supplement, as well as associated exposure/risk analyses, the 1990 Staff Paper recommended that the EPA Administrator consider options for revision of the Pb NAAQS to a level in the range of 0.5 to 0.75 $\mu g/m^3$ (30-day arithmetic mean). After consideration of evaluations contained in the above documents, EPA chose not to propose revision of the Pb NAAQS. As part of implementing a broad, integrated Strategy for Reducing Lead Exposures (U.S. Environmental Protection Agency, 1991), the Agency focused efforts primarily on regulatory or remedial clean-up actions aimed at reducing Pb exposures from a variety of non-air sources judged to pose more extensive public health risks to U.S. populations as well as on other actions to reduce Pb emissions to air.

Changes in relative contributions of various Pb sources and exposure pathways to human exposures in the United States, and EPA actions to reduce such exposures, thusly provide very important background context for this current Pb criteria and NAAQS review. Since 1978, the amount of Pb emitted into the air nationally has markedly declined. For example, as illustrated in Chapters 2 and 3 of this document, from 1982 to 2002 Lead emissions into the air decreased by 93%, and the average air quality concentration of Pb decreased by 94% from 1983 to 2002 (http://www.epa.gov/airtrends/lead2.html). Total Pb emissions into the air decreased from about 220,000 tons in 1970 to less than 4,000 in 1999. This decline is mainly attributable to EPA's regulatory actions that led to notable reductions in the content of Pb in gasoline (see, for example, 50 FR 9386), which substantially altered basic patterns of air Pb emissions in the

United States (http://www.epa.gov/airtrends/lead2.html). Emissions from stationary sources have also been greatly reduced (http://www.epa.gov/airtrends/lead2.html, Figure 2-11); but, given the even greater reductions in emissions from transportation sources, industrial processes (including smelters and battery manufacturers) now constitute a larger percentage of remaining Pb emissions to the atmosphere (http://www.epa.gov/airtrends/lead2.html, Figure 2-12).
In short, Pb emissions into the atmosphere decreased greatly in the 1980's and 1990's, a trend that has continued through to the present. Consequently, airborne Pb now represents only a relatively small component of total exposure to Pb in the United States, such that the principal sources and pathways for U.S. Pb exposure among the classically-defined most sensitive population group (young children) involve non-inhalation pathways, e.g., ingestion of Pb from deteriorating paint, dust, historically contaminated soil, drinking water, and food. While these downward trends in air Pb exposures nationwide are encouraging, several important sources of air Pb exposure may still persist in some localities. Lead emissions from specific stationary sources and/or reentrainment of Pb-contaminated soils (including from past deposition of airborne Pb) may still have significant impacts on a local level. Recognition of the multimedia nature of Pb exposure of the general population has been important historically and sorting out relative contributions to total Pb exposure burdens represents an important input to the current periodic Pb NAAQS review effort.

Since the 1980's, EPA has played a major, effective role in working to reduce the main sources of Pb exposure for most children, including deteriorating Pb-based paint, Pb-contaminated dust, and Pb-contaminated residential soil (http://www.epa.gov/lead/). For example, EPA has established standards for Pb-based paint hazards and Pb dust cleanup levels in most pre-1978 housing and child-occupied facilities, and is now developing standards for those conducting renovation activities that create Pb-based paint hazards and for the management and disposal of Pb-based debris (http://www.epa.gov/lead/regulation.htm). In addition, EPA has developed standards for management of Pb in solid and hazardous waste, continues to oversee the cleanup of Pb contamination at Superfund facilities, and issued regulations to reduce Pb in drinking water (http://www.epa.gov/lead/sources.htm). Beyond taking specific regulatory actions, the Agency’s Lead Awareness Program also continues to work to protect human health and the environment against the dangers of Pb by conducting research and designing educational outreach efforts and materials (http://www.epa.gov/lead/).

Since the 1980's, EPA has also promulgated regulations under Section 112 of the Clean Air Act (42 U.S.C. § 7412), to address emissions of Pb components and other toxic pollutants from both primary Pb smelters and secondary Pb smelters (40 CFR Subparts X and TTT). Under section 112(d), these emission standards are to require "the maximum degree of reduction in emissions" that are "achievable." Thus, EPA promulgated section 112(d) standards for secondary Pb smelters on June 23, 1995 (60 Fed. Reg. 3587) and revised them on June 13, 1997 (62 Fed. Reg. 32209), followed by promulgation of section 112(d) standards for primary Pb smelters on June 4, 1999 (64 Fed. Reg. 30194).

1.2 CURRENT LEAD CRITERIA AND NAAQS REVIEW

1.2.1 Procedures and Key Milestones for Document Preparation

It is important to emphasize at the outset that development of the present revised Lead AQCD included substantial external (non-EPA) expert inputs and opportunities for public input through (a) public workshops involving the general scientific community, (b) several iterative reviews of successive drafts of this document by CASAC, and (c) comments from the public on successive drafts. Such extensive external inputs received via these mechanisms help to ensure that the review of the Pb NAAQS will be based on critical assessment in this document of the latest available pertinent science.

The approach used for developing this revised Lead AQCD has built on experience derived from other recent criteria document preparation efforts. This includes close coordination between NCEA-RTP and OAQPS staff, as well as with others, throughout the document preparation and review process. Briefly, the respective responsibilities for production of the document and meeting key milestones were as follows. An NCEA-RTP Lead Team was designated as being responsible for creation and implementation of a project plan for developing the Lead AQCD, taking into account input from individuals in other ORD units, OAQPS, and other EPA program/policy offices identified as part of the EPA Lead Work Group. The Lead Team defined critical issues and topics to be addressed by the authors and provided direction in order to focus on evaluation of those studies most clearly identified as likely being important for U.S. air standard setting purposes. Criteria document materials were authored in part by NCEA-RTP Lead Team staff with appropriate expertise in particular areas and by non-EPA

consultants to EPA who are recognized experts in pertinent specific areas (e.g., Pb biokinetic modeling, toxicology, and epidemiology).

Key milestones for development of this Lead AQCD are listed in Table 1-1. As a first step, EPA announced on November 9, 2004 the official initiation of the current periodic review of Lead air quality criteria.

Table 1-1. Key Milestones and Projected Schedule for Development of Revised Lead Air Quality Criteria Document (Lead AQCD)

Major Milestones	Target Dates
1. Literature Search	Ongoing
2. Federal Register Call for Information	November 9, 2004
3. Prepare Draft Lead AQCD Project Work Plan	Nov-Dec 2004
4. Release Draft Project Plan for Public Comment/CASAC Review	January 2005
5. Public Comment Period	Jan/Feb 2005
6. CASAC Teleconsultation on Project Work Plan	March 28, 2005
7. Workshop Drafts of Lead AQCD Chapters	May/June 2005
8. Peer Consultative-Review Workshop(s)	July/August 2005
9. Release First External Review Draft	December 1, 2005
10. Public Comment Period	Dec 2005-Feb 2006
11. CASAC/SAB Public Review Meeting *(First Ext. Rev. Draft)*	Feb. 28-Mar 1, 2006
12. Release Second External Review Draft	May 2006
13. Public Comment Period	May/June 2006
14. CASAC/SAB Public Review Meeting *(Second Ext. Rev. Draft)*	June 28-29,2006
15. Release Revised Drafts of Executive Summary and Integrative Synthesis Chapter for Public Comment and CASAC Review	July 31, 2006
16. CASAC Review of Executive Summary and Integrative Synthesis via Public Teleconference	August 15, 2006
17. Final Lead AQCD	October 1, 2006[1]

[1] Court-ordered deadline for EPA to produce a final Lead AQCD in relation to Missouri Coalition for the Environment v. EPA, Civil Action No. 4:04-CV-00660 (ERW) (E.D. Mo. Sept. 14, 2005). Also, note that materials contributed by non-EPA authors, at times, were modified by EPA staff in response to internal and/or external review comments and that EPA is responsible for the ultimate content of this Lead AQCD.

More specifically, under processes established in Sections 108 and 109 of the Clean Air Act, U.S. EPA began by announcing in the Federal Register (69 FR 64,926) the formal commencement of the current review process with a call for information (see Federal Register, 2004). In addition, EPA prepared a January 2005 draft Lead AQCD Work Plan that was made available for public comment and was the subject of teleconsultation with CASAC on March 28, 2005 as a means by which to communicate the process and timeline for development of a revised Lead AQCD. Next, expert consultants to NCEA-RTP and NCEA-RTP staff (a) carefully evaluated pertinent new studies obtained via the call for information and via ongoing literature searches conducted by NCEA-RTP information retrieval specialists and (b) prepared preliminary draft chapter materials for inclusion in this revised Lead AQCD. Those preliminary draft materials then underwent expert peer discussion at public workshops organized and conducted by NCEA-RTP in July/August, 2005. After consideration of comments received at the workshops, appropriate revisions were made in the draft materials and incorporated into the First External Review Draft of the Lead AQCD, which was made available for public comment (for 90 days) and CASAC review at a public meeting on February 28-March 1, 2006. EPA, taking into account CASAC and public comments, then incorporated revisions into the draft AQCD before releasing a Second External Review Draft (May 2006) of it for further review by the public and by CASAC at a public meeting held June 28-29, 2006. Revised drafts of the Executive Summary and the Integrative Synthesis were next quickly prepared and made available on August 1, 2006 for public comment and CASAC teleconference on August 15, 2006. Further revisions were then incorporated, in response to the last two public comment and CASAC reviews, to complete the final version of this revised Lead AQCD for issuance by October 1, 2006. The final document (dated October 2006) was then published, and its availability to the public announced in the Federal Register.

Drawing upon evaluations in this Lead AQCD and other Pb exposure/risk analyses, an associated Lead Staff Paper prepared by EPA's Office of Air Quality Planning and Standards (OAQPS) assesses policy implications of key information in the Lead AQCD, reports pertinent exposure and risk analyses, and poses possible options for the EPA Administrator to consider regarding whether to retain or, if appropriate, revise the Pb NAAQS. Taking into account CASAC and public comments, the EPA Administrator will consider the options posed in the Staff Paper; propose decisions regarding possible retention or revision of the primary and/or

secondary Pb NAAQS via the Federal Register for public comment; and then, after consideration of comments received, promulgate final Pb NAAQS review decisions.

1.3 ORGANIZATIONAL STRUCTURE AND CONTENT OF THE DOCUMENT

1.3.1 Ascertainment of Literature and General Document Format

Lists of references published since completion of the 1986 Lead AQCD/Addendum and 1990 Supplement were made available to the authors. The references were mainly selected from information data base (e.g., Pub Med) searches conducted by EPA. However, additional references have been added as work has proceeded in creating the present Lead AQCD materials. As an aid in selecting pertinent new literature, the authors were also provided with a summary of issues to be addressed in this revised Lead AQCD. Many such issues identified in the course of previous Lead criteria assessments, through interactions between EPA Lead Team and Lead Work Group members, and via workshop discussions.

The general format used in this document is to open each new chapter (or main section) for the updated Lead AQCD with concise summary of key findings/conclusions from previous Lead criteria assessments, especially the 1986 Lead AQCD/Addendum (U.S. Environmental Protection Agency, 1986a,b) and 1990 Supplement (U.S. Environmental Protection Agency, 1990). After presentation of such background information, the remainder of each chapter or section typically provides an updated discussion of newer literature and resulting key conclusions. In some cases where no new information is available, the summary of key findings and conclusions from the previous Pb criteria assessment(s) must suffice as the basis for current key conclusions. Increased emphasis is placed in the main chapters of this revised Lead AQCD on interpretative evaluation and integration of evidence pertaining to a given topic than was typical of many previous EPA air quality criteria documents, with more detailed descriptions of individual studies or other supportive information being provided in a series of accompanying annexes.

1.3.2 Organization and Content of the Document

This updated Lead AQCD critically assesses scientific information on health and welfare effects associated with exposure to the concentrations of Pb in ambient air. The document is not intended to be a detailed, exhaustive review of the literature. Rather, the cited references reflect the current state of knowledge regarding important issues pertinent to decisions regarding possible revision by EPA of the Pb NAAQS. Although emphasis is placed mainly on the discussion of health and welfare effects data, other scientific information also is evaluated in order to provide a better understanding of the nature, sources, distribution, and concentrations of Pb in ambient air, as well as the measurement of human exposure to Pb.

The focus of discussion is on assessment of selected pertinent scientific information newly published since the last prior assessments of air quality criteria for Pb contained in the 1986 Lead AQCD/Addendum and 1990 Supplement. As noted earlier, key findings and conclusions from the 1986 Lead AQCD/Addendum and 1990 Supplement are typically first briefly summarized at the outset of discussion of a given topic, with appropriate reference back to the previous criteria assessment materials. Typically, important prior studies are more specifically discussed only if they are open to reinterpretation in light of newer data and/or are judged to be potentially useful in decisions on revision of the Pb NAAQS. Generally, only information that has undergone scientific peer review and has been published (or accepted for publication) in the open literature through December 31, 2005 has been considered in this revised Lead AQCD. Certain other unpublished analyses (e.g., EPA analyses of recently available U.S. Lead air quality data) are also considered, depending on the importance of the subject information and its pertinence to criteria development for Pb NAAQS, as determined in consultation with CASAC.

This Lead AQCD consists of two volumes. The first volume (Volume I) includes eight chapters that comprise the main body of the revised Lead AQCD and an Executive Summary for all chapters. In Volume I, this introductory chapter (Chapter 1): (a) provides brief statements regarding the purpose of the document; (b) presents information on the legislative background and regulatory chronology of Pb criteria reviews; and (c) presents an overview of the organization of the document. Chapter 2 discusses the physics and chemistry of Pb, as well as sources, emissions, transport and deposition/fate. Chapter 3 next provides information on environmental concentrations, dispersal patterns, and multimedia exposure pathways. Chapter 4

focuses on the measurement of concentrations of Pb in biological samples and the modeling of multimedia exposure impacts on human internal Pb burdens, especially as indexed by blood Pb or bone Pb concentrations. Then, Chapter 5 discusses toxicologic studies of Pb health effects in humans, laboratory animals, and in vitro test systems; whereas Chapter 6 assesses Pb-related epidemiologic (observational) studies of human population groups. Chapter 7 deals with ecological and other environmental effects of Pb. Lastly, Chapter 8 provides an overall integrative synthesis of key information drawn from the earlier chapters to delineate: human Pb exposure pathways and trends; health effect findings and conclusions of most importance for derivation of primary Pb NAAQS; and key types of welfare effects (in this case, ecosystem) findings pertinent to the derivation of secondary Pb NAAQS. Volume II of this revised Lead AQCD includes several annexes containing detailed descriptive materials supporting the more interpretative evaluations presented in several of the main chapters dealing with Pb-related health and/or ecological effects.

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2. CHEMISTRY, SOURCES, AND TRANSPORT OF LEAD

The purpose of this chapter is to provide background information on the chemical properties of lead (Pb) that are relevant to its transport within the environment, its transport into ecosystems and its impact on human health; to discuss the known sources of Pb in the environment; and to outline the mechanisms by which Pb is transported within the environment. The chapter does not provide a comprehensive list of all sources of Pb, nor does it provide emission rates or emission factors for all source categories, since such information is available for only a limited number of sources. Rather, the chapter provides data on the chemistry, sources, and transport of Pb where information is available in the literature or via publicly accessible EPA databases, websites, and reports. Particle size distribution data for Pb are even scarcer than information on total Pb emissions from sources; but particle size data are presented where such data are available.

2.1 PHYSICAL AND CHEMICAL PROPERTIES OF LEAD

Properties of Elemental Lead

Elemental Pb possesses an array of useful physical and chemical properties, making it among the first metals to be extracted and used by humankind. It has a relatively low melting point (327.5℃), is a soft, malleable, and ductile metal, a poor electrical conductor, and is easily cast, rolled and extruded. Although sensitive to environmental acids, after exposure to environmental sulfuric acid (H_2SO_4), metallic Pb becomes impervious to corrosion due to weathering and submersion in water. This effect is due to the fact that Pb lead sulfate ($PbSO_4$), the relatively insoluble precipitate produced by reaction of Pb with H_2SO_4, forms a protective barrier against further chemical reactions (Schweitzer, 2003). This aspect of its chemistry made Pb especially convenient for protective surface coatings (e.g. paint), roofing, containment of corrosive liquids, and (until the discovery of its adverse health effects), construction of water supply systems.

Lead is readily extracted from *galena*, a widely available lead sulfide mineral form (PbS), by froth flotation, followed by roasting in the presence of a limited amount of oxygen to form *litharge*, one of two forms of lead oxide (PbO). Elemental Pb is then isolated by reducing PbO by way of heating in the presence of elemental carbon (coke, charcoal) (Greenwood and Earnshaw, 1984). This and other extraction and recovery processes are discussed in greater detail later in this chapter.

Lead alloys constitute 60% of Pb used in industry (Prengaman, 2003). The major alloying elements are antimony, calcium, tin, copper, tellurium, arsenic, and silver. Selenium, sulfur, bismuth, cadmium, indium, aluminum, and strontium are also sometimes used. Lead alloys are found primarily in Pb acid batteries, solder, ammunition, and cable sheathing (Prengaman, 2003). Table 2-1 provides a list of Pb alloys in wide use by industry.

Some of the physical properties of elemental Pb are listed in Table 2-2. The most important of these properties, when evaluating the transport routes for Pb within the atmosphere, is its boiling point. As indicated, Pb will only exist in the vapor phase at or above 1750°C. Therefore, at ambient atmospheric temperatures, elemental Pb will deposit to surfaces or exist in the atmosphere as a component of atmospheric aerosol.

Oxidation States of Lead

Lead is the heaviest congener of carbon and shares many properties with the other elements found in the same column of the periodic chart (silicon, germanium, and tin). As Group IV elements, these elements have four valence electrons (2 *p* and 2 *s*), allowing for both divalent and tetravalent compounds.

Due to its high atomic number (82), the valence electron orbitals of the Pb atom exist at a comparatively large distance from its nucleus. As with *s* and *p* orbitals at any quantum level, electrons in the 6*s* orbital tend to occupy space near the nucleus with greater probability than those in the 6*p* orbital. The strong attraction produced by the large Pb nucleus, combined with the long distance that the 6s electrons must travel, result in electron accelerations to relativistic speeds. The Theory of Relativity states that as the velocity of matter approaches the speed of light, its apparent mass increases. In this instance, the electrons in the Pb 6*s* orbital experience an increase in weight, which increases the attractive effect of the positive nuclear charge, which contracts the diameter of the Pb 6*s* orbital (Pitzer, 1979). This "relativistic effect" on valence

Table 2-1. Lead Alloys and Their Industrial Applications

Pb Alloy	Uses
Pb-Antimony	Grids, posts, and connectors for Pb-acid batteries, ammunition, cable sheathing, anodes, tank linings, pumps, valves, and heating and cooling coils
Pb-Calcium	Automotive, standby power, submarines, and specialty sealed batteries, electrowinning anodes, cable sheathing, sleeving, specialty boat keels, and Pb alloy tapes
Pb-Tin	Soldering for electronics, general purposes, automobile radiators, and heat exchangers, corrosion resistant coatings on steel and copper, cable sheathing, fuses, sprinkler system alloys, foundry pattern alloys, molds, dies, punches, cores, mandrels, replication of human body parts, and filters for tube bonding
Pb-Copper	Pb sheet, pipe, cable sheathing, wire, fabricated products, tank linings, tubes for acid-mist precipitators, steam heating pipes for sulfuric acid or chromate plating baths, and Pb sheathing for roofs
Pb-Silver	Anodes, high-temperature solders, insoluble anodes in the electrowinning of zinc and manganese, and soft solders
Pb-Tellurium	Pipes, sheets, shielding for nuclear reactors, and cable sheathing
Pb-Bismuth	Fuses, sprinkler system alloys, foundry pattern alloys, molds, dies, punches, cores, mandrels, solders, replication of human body parts, and filters for tube bonding
Pb-Cadmium	Fuses, sprinkler system alloys, foundry pattern alloys, molds, dies, punches, cores, mandrels, solders, replication of human body parts, and filters for tube bonding
Pb-Indium	Fuses, sprinkler system alloys, foundry pattern alloys, molds, dies, punches, cores, mandrels, solders, replication of human body parts, filters for tube bonding, and joining metals to glass
Pb-Strontium	Battery grids
Pb-Lithium	Bearings, Pb-acid battery grids
Pb-Antimony-Tin	Printing, bearings, solders, slush castings, and specialty castings
Pb-Calcium-Aluminum	Negative battery grids of Pb-acid batteries
Pb-Calcium-Tin	Positive grids of Pb-calcium batteries, and Pb anodes for electrowinning
Pb-Calcium-Silver	Zinc electrowinning
Pb-Antimony-Silver	Anodes used for the production of thin copper foil in electronics, and anodes in cathodic protection of steel pipes and structures in water
Pb-Silver-Tin	Anodes in cathodic protection of steel pipes and structures in water, and soft solders
Pb-Strontium-Tin	Anodes for copper electrowinning
Pb-Lithium-Tin	Pb-acid battery grids

Source: Prengaman (2003).

Table 2-2. Physical Properties of Elemental Lead

Physical Property	
Atomic number	82
Atomic weight	207.2
Valence electrons	$[Xe]4f^{14}5d^{10}6s^{2}6p^{2}$
Melting point	328 °C
Boiling point	1750 °C
Density	11.34 g/cm^3
Atomic radius	146 pm
Standard reduction potential	−0.126V
Oxidation numbers	+2, +4
Ionization Energy	715.6 kJ/mol

Source: Kotz and Purcell (1991).

electrons are proportional to the square of atomic number, and manifests within the Group IV elements as a distinctly increasing trend in the stability of the divalent state from Si down to Pb. In the case of Pb, the two 6*s* electrons behave as if they were chemically inert, leaving only the two 6p electrons available for bonding or oxidation under ordinary conditions. For this reason, the relativistic effect is also known as the "inert pair effect." Consequently, Pb(II) is the most common oxidation state in which Pb is found in the environment (King, 1995; Claudio et al., 2003).

Lead is distinguished from other elements that are subject to relativistic effects by its preference for forming tetravalent (Pb(IV) organometallic compounds. However, in fact, it is only with rare exception that Pb(II) organometallic compounds form (Pelletier, 1995; Greenwood and Earnshaw, 1984). All simple alkyllead compounds, such as the well-known fuel additives, tetramethyllead (TML) and tetraethyllead (TEL) are composed of Pb(IV). In contrast, inorganic Pb(IV) compounds (such as PbO_2) are strong oxidants and unstable with respect to their Pb(II) analogs. There are, overall, more than 200 known organolead compounds (Harrison, 1985).

In relation to the other Group IV metals, however, Pb forms the least stable and most reactive organometallic derivatives. This is largely due to the weak bond between Pb and

carbon, consistent with its large atomic size, and the influence of the relativistic effect on its valence orbitals. Specifically, the mean bond dissociation energies of the metal-carbon bonds for Group IV elements are 56.7 kcal/mol for germanium, 46.2 kcal/mol for tin, and 30.8 kcal/mol for Pb (Shapiro & Frey, 1968). Organolead compounds are thermally unstable and will decompose to metallic Pb and free radicals at relatively low temperatures (Willemsens and van der Kerk, 1965). For example, TML decomposes at temperatures above 200°C, and TEL decomposes at temperatures above 110°C (King, 1995). In solution, organolead compounds decompose in the presence of UV radiation (1 hr/254 nm) and sunlight (Gomez Ariza et al., 2000).

Tetralkyllead compounds have atmospheric residence times ranging from a few hours to a few days (Pelletier, 1995). TML and TEL react with OH in the gas-phase, following pseudo-first order kinetics, to form a variety of products that include ionic trialkyllead (TriAL), dialkyllead (DiAL) and metallic Pb. Trialkyllead is slow to react with OH and is quite persistent in the atmosphere (Hewitt and Harrison, 1986; Harrison and Laxen, 1980).

Lead Oxides, Chalcogenides, and Salts

A rich variety of inorganic Pb compounds and complex salts can be prepared in the laboratory under conditions of temperature and pressure not usually seen in the environment. Information on the many possible organic and inorganic Pb compounds can be found in the text by Greenwood and Earnshaw (1984). Several representative Pb salts and oxides are described in Tables 2-3 and 2-4. Inorganic Pb compounds that can be found in the environment are the main focus of this discussion.

As explained earlier, Pb exists preferentially in its +2 oxidation state in the environment. Under aqueous acidic conditions, Pb readily oxidizes, with a strongly positive electrochemical potential ($E^0 = 1.355$ V) and a large equilibrium constant ($K = 10^{91.6}$), to form Pb(II) (Singley, 1994):

$$2Pb + O_2 + 4H^+ \rightarrow 2Pb^{2+} + 2H_2O \qquad (2\text{-}1)$$

Table 2-5 lists the various Pb compounds and salts that are present naturally or are introduced into the environment by anthropogenic activities. From this list, it is clear that only a relatively limited number of salts and covalently-bound Pb compounds are of significance in

Table 2-3. Lead Salts: Names, Formulae, Physical Characteristics, and Uses

Category	Compound Name	Formula	Form	Uses
Pb Acetates	Anhydrous Pb Acetate	$Pb(C_2H_3O_2)_2$	White, crystalline solid	Preparing other Pb salts.
	Basic Pb Acetate	$Pb(C_2H_3O_2)_2 \cdot 2Pb(OH)_2$	Heavy, white powder	Sugar analysis.
	Pb Acetate Trihydrate	$Pb(C_2H_3O_2)_2 \cdot 3H_2O$	White, monoclinic crystalline solid	Making other Pb compounds, mordant for cotton dyes, water repellant, processing agent for cosmetics, perfumes, and toiletries.
	Pb Tetraacetate	$Pb(C_2H_3O_2)_4$	Colorless, monoclinic crystalline solid	Oxidizing agent in organic synthesis, cleaving of a-hydroxy acids, introducing acetyl groups in organic molecules.
Pb Carbonates	Pb Carbonate	$PbCO_3$	Colorless, orthorhombic crystals	Catalytic polymerization of formaldehyde, improving the bonding of polychloroprene to metals in wire-reinforced hoses, a component of high-pressure lubricating greases, and a lubricant for polyvinyl chloride.
	Basic Pb Carbonate	$(Pb(CO_3)_2)_2 Pb(OH)_2$	White, hexagonal crystals	Ceramic glazes, a curing agent with peroxides to form polyethylene wire insulation, a color-changing component of temperature-sensitive inks, a component of lubricating grease, and a component of weighted nylon-reinforced fish nets made of polyvinyl chloride fibers.
Pb Halides	Pb Fluoride	PbF_2	Colorless, orthorhombic crystals	Glass sealing disks for IR sensors, wear-resistant automotive shock absorbers, electrolytic deposition of Pb, flux for brazing of aluminum and its alloys, optical glass fibers for IR transmission, and thin film batteries.
	Pb Chloride	$PbCl_2$	White, orthorhombic needles	Artist's pigment, precursor of organolead compounds, seawater-activated batteries, expanding polymer mortar, flux for soldering cast iron and cast brass, sound-insulating rubber sealants, corrosion inhibitor for galvanized steel, and infrared-transmitting glasses for CO_2 lasers.
	Pb Bromide	$PbBr_2$	White, orthorhombic crystals	Filler for flame-resistant polypropylene, glass optical waveguides for infrared thermometers and catalysts for producing polyesters.
	Pb Iodide	PbI_2	Powdery, yellow, hexagonal crystals	Aerosols for cloud seeding, making high-contrast photographic images of laser radiation, high capacity cathodes in lithium batteries, and low-temperature thermographic copying materials.
Pb Silicates	Pb Monosilicate	$3PbO \cdot 2SiO_2$	White, trigonal crystalline powder	Formulating Pb-bearing glazes for ceramics, source of PbO in glass manufacturing.
	Pb Bisilicate	$PbO\ 0.03Al_2O_3 \cdot 1.95SiO_2$	Pale yellow powder	Ceramic glazes.
	Tribasic Pb Silicate	$3PbO \cdot SiO_2$	Reddish-yellow powder	Glass and frit production.
Pb Sulfates	Tribasic Pb Sulfate	$3PbO\ PbSO_4\ H_2O$	Fine, white powder	Providing long-term heat stability to PVC, electrical insulation, activation for azodicarbonamide blowing agents for vinyl foam.

Source: Carr (2003).

Table 2-4. Lead Oxides: Names, Formulae, Physical Characteristics, and Uses

Name	Formula	Form	Uses
Pb Monoxide	PbO	Reddish below 489°C, yellow at high temperatures	Pastes for the grids of Pb-acid batteries, optical, electrical, and electronic glasses, glazes for fine tableware, vulcanizing agent for rubber, Pb soaps used in driers as varnishes, high-temperature lubricants, neutralizing agent in organic synthesis, heat stabilizer in plastics, and starting material in the production of pigments.
Pb Dioxide	PbO_2	Brownish-black crystalline powder of fine flakes	Active material of the positive plates in Pb-acid batteries, oxidizing agent in the manufacture of chemicals, dyes, matches, pyrotechnics, and liquid polysulfide polymers, antifriction agent for plastic sliding bearings, ballistic modifiers in high-energy propellants, electrodes for seawater electrolysis, filters for desulfurization of waste gases, vulcanizing agents for butyl-rubber puncture-sealing layers inside tires.
Pb Sesquioxide	Pb_2O_3	Amorphous, orange-yellow powder	Ballistic modifier for high-energy propellants, cathode material in lithium batteries, additive to increase the shattering force of explosives.
Red Pb	Pb_3O_4	Brilliant orange-red pigment	Pigment in anticorrosion paints for steel surfaces, Pb oxide pastes for tubular Pb-acid batteries, ballistic modifiers for high-energy propellants, ceramic glazes for porcelain, lubricants for hot pressing metals, radiation-shielding foam coatings in clinical x-ray exposures, and rubber adhesives for roadway joints.

Source: Carr (2003).

the environment, i.e., sulfates ($PbSO_4$), chlorides ($PbCl_2$), carbonates ($PbCO_3$, $Pb(HCO_3)_2$), hydroxides ($Pb(OH)_2$), nitrates ($Pb(NO_3)_2$), phosphates ($PbPO_4$, $Pb(HPO_4)_2$), silicates, oxides (PbO, Pb_3O_4), and PbS. With the exception of the covalently-bound sulfide and oxide, these compounds are derived from acids (or the related anions) that are common in the environment, such as sulfuric acid (H_2SO_4), nitric acid $(HNO_3)^-$, phosphoric acid (H_3PO_4), and carbonic acid (H_2CO_3), an acid that forms when CO_2 dissolves in water. Lead salts, once formed, tend to be only slightly soluble in neutral solutions, but are quite soluble in the presence of acid (Weast et al., 1988).

Table 2-5. Lead Compounds Observed in the Environment

Location	Observed Pb Compounds
Minerals	PbS (*Galena*) PbO (*Litharge*, *Massicot*) Pb_3O_4 (*Minium or "Red Lead"*) $PbCO_3$ (*Cerussite*) $PbSO_4$ (*Anglesite*)
Smelting Aerosols	Pb^0, PbS $PbSO_4$, PbO, $PbSO_4$.PbO $PbCO_3$ Pb silicates
Coal Combustion Aerosols	PbS PbSe
Coal Combustion Flue Gases	Pb^0, PbO, PbO_2 (*Above 1150K*) $PbCl_2$ (*Low rank coals, above 1150K*) $PbSO_4$ (*Below 1150 K*)
Wood Combustion	$PbCO_3$
Waste Incineration Aerosols	$PbCl_2$ PbO
Soils Near Mining Operations	$PbCO_3$ $PbSO_4$ $[PbFe_6(SO_4)_4(OH)_{12}]$ $[Pb_5(PO_4)3Cl]$ $[Pb_4SO_4(CO_3)_2(OH)_3]$ $PbS\text{-}Bi_2S_3$ Pb oxides, silicates
Motor vehicle exhaust (combustion of leaded fuel)[a]	PbBrCl $PbBrCl\text{-}2NH_4Cl$ $PbBrCl\text{-}NH_4Cl$
Roadside dust[a]	$PbSO_4$, Pb^0, $PbSO_4$ $(NH_4)SO_4$, Pb_3O_4, $PbO\text{-}PbSO_4$ and $2PbCO_3\text{-}Pb(OH)_2,PbSO_4$
Other mobile sources:	
Brake wear, wheel weights	Pb^0
NASCAR vehicle emissions	Pb halides
Aircraft engine wear	Pb^0
Lawn mowers	Pb halides (*Battery leakage*)

[a]Source: Biggins and Harrison (1979, 1980).

Lead Coordination Chemistry, and Its Role in Biochemistry

The formation of coordinate covalent complexes represents a different class of chemical interaction from the formation of simple covalent compounds and salts. "Coordinate covalent" bonds form when anions or neutral molecules interact with metal ions in solution that are capable

of donating both of the electrons required to form a covalent bond. These molecules (or anions) are called "ligands" or "electron donors." Ligands possess a filled valence orbital with a geometry that allows it to overlap to a substantial degree with an empty orbital associated with the metal ion. In the case of Pb, its large atomic size is associated with several out-lying empty atomic orbitals leading to a tendency to form a large number of coordinate covalent bonds (Claudio et al., 2003). This is suggested by the coordination number (9) of $PbCl_2$, in its crystalline form, which is able to share electrons with 9 adjacent chloride ions (Cl^-) (Douglas et al., 1983).

Molecules capable of serving as ligands for metal ions in solution take many forms. "Monodentate" ligands are molecules capable of providing 2 electrons to form a single coordinate bond, such as water (H_2O), ammonia (NH_3); "multidentate" ligands can participate in more than one coordinate bond. A common term for the binding of a metal ion by a multidentate ligand is "chelation." The chelating agent, ethylenediaminetetraacetic acid (EDTA), is a well known hexadentate ligand, containing 6 functional groups capable of forming 6-coordinate bonds with metal ions in aqueous solution. Proteins, particularly the active sites of enzymes, contain functional groups—usually associated with amino acid side chains—that can serve as ligands for metal ions. In fact, the zinc finger proteins must form coordinate complexes with Zn^{2+} ions to stabilize their active conformations (Claudio et al., 2003).

Several types of equilibrium constants for ligand-metal interactions can be derived, depending on the property of interest. One formulation, the "binding constant (K_b)," between the free metal ion and ligands in- solution with the ligand-metal complex, is derived below for a negatively charged ligand:

$$K_b = \text{binding constant} = \frac{[ML_x{}^{n-1}]}{[ML_{x-1}{}^{n+}][L^-]} \tag{2-2}$$

Where:

$$K_{b1} = \frac{[ML^{n-1}]}{[M^{n+}][L^-]};$$

$$K_{b2} = \frac{[ML_2{}^{n-2}]}{[ML^{n-1}][L^-]};$$

Etc.

K_{b1} provides a measure of the stability of a solution of the free metal ion, M^{n+} and an individual ligand, L, compared to the complex of ML^{n+}. Alternatively, K_{b1} gives an indication of the strength of the interaction between M^{n+} and L. Thus, K_{b2} indicates the strength of the interaction between the ML^{n+} complex and an additional ligand, L. Subsequent additions of ligands to the complex are described following the same convention. Binding constants are useful, in particular, for evaluating the strength of interactions between metals and small (monodentate) ligands. The form typically used to evaluate binding between metals and proteins is the "dissociation" constant, K_d. The example given here is for a neutral ligand:

$$K_d = \text{dissociation constant} = \frac{[ML_{x-1}^{n+}]\,[L^{\circ}]}{[ML_x^{n+}]} \qquad (2\text{-}3)$$

Where:

$$K_{d1} = \frac{[M^{n+}][L^{\circ}]}{[ML^{n+}]};$$

$$K_{d2} = \frac{[ML^{n+}][L^{\circ}]}{[ML_2^{n+}]};$$

Etc.

K_d is the inverse of K_b, in that it refers to stability of the existing complex between ligand and metal, versus the free metal ion and the free ligand. K_{d1} is a measure of the strength of the bond between an individual ligand and the metal-ligand complex. K_{d2} indicates the strength of the interaction as the second ligand is, subsequently, removed. A variety of quantitative, analytical methods are available for measuring the binding and dissociation constants for specific combinations of metals and ligands.

A simple, qualitative model commonly used for discussing the relative strength of coordinate covalent bonding between different metals and ligands is the Pearson's Hard-Soft Acid-Base (HSAB) model (Douglas et al., 1983). Heavier metals, such as Pb, which have more electrons and more spatially diffuse valence orbitals, are described as "soft" (Lewis) acids. Lighter metals, with fewer electrons and more closely-spaced valence orbitals, are described as "hard" (Lewis) acids. These metals tend to preferentially bond with ligands having similar electronic properties. Hard acids tend, for example, to prefer oxygen-based ligands, i.e.,

"hard bases," and soft acids prefer ligands based on larger atoms, such as sulfur and selenium, i.e., "soft bases."

The HSAB concept is useful for understanding the behavior of Pb in the biological context. Lead forms coordinate covalent bonds with ligand atoms with effectiveness that declines with atomic size. For example, Pb forms especially stable bonds with sulfur and sulfur-containing compounds, but somewhat less so with carboxylic acids (O-based ligands) and imidazoles (N-based ligands) (Claudio et al., 2003).

In biological systems, Pb competes very effectively with native or homeostatic metal ions for binding with the sulphahydryl, carboxyl and imidazole side-chains comprising enzyme active sites. This competition leads to inhibition of enzyme activity, as well as the replacement of calcium in bone and, ultimately, to a substantial list of negative human health effects. The relative strength of these different interactions appears to be reasonably well-predicted by the HSAB model.

By far, the most effective biological ligands for Pb are amino acid side-chains containing sulfur and selenium. Smaller electron donors (hard bases), such as carboxylic acids that bind Pb via electrons associated with oxygen, form weaker bonds. These complexes are generally more labile, i.e., bonds form and break rapidly, thus allowing more effective competition at protein binding sites amongst metals available in solution. Simple ligands that are examples of this case are the amine functional group, -NH, and the thiol functional group, -SH. The amine group has a Pb binding constant on the order of 100, whereas the thiol group binding constant is on the order of 10^7. Example proteins in this instance are carboxypeptidase A, a zinc-binding protein, with carboxylate and histidine side-chains, and the four cysteine zinc finger consensus peptide, CP-CCC. Carboxypeptidase A has a Pb dissociation constant of approximately 10^{-4} M, versus that of the zinc finger protein, which is 3.9×10^{-14} M. Claudio et al. (2003) concluded, on the basis of these values, that carboxypeptidase A is unlikely to be a protein associated with Pb poisoning, while cysteine-rich proteins, including the zinc enzyme, d-aminolevulinic acid dehydratase (ALAD), the second enzyme in the heme biosynthetic pathway, are more likely targets. ALAD active site, with its Cys_3 active site, is known to be inhibited at femtomolar (10^{-15} M) concentrations of Pb in vitro.

Figure 2-1 illustrates the wide array of possible inhibitory interactions between Pb^{2+} and proteins responsible for transduction at nerve synapses. Targets for Pb^{2+} interference at the

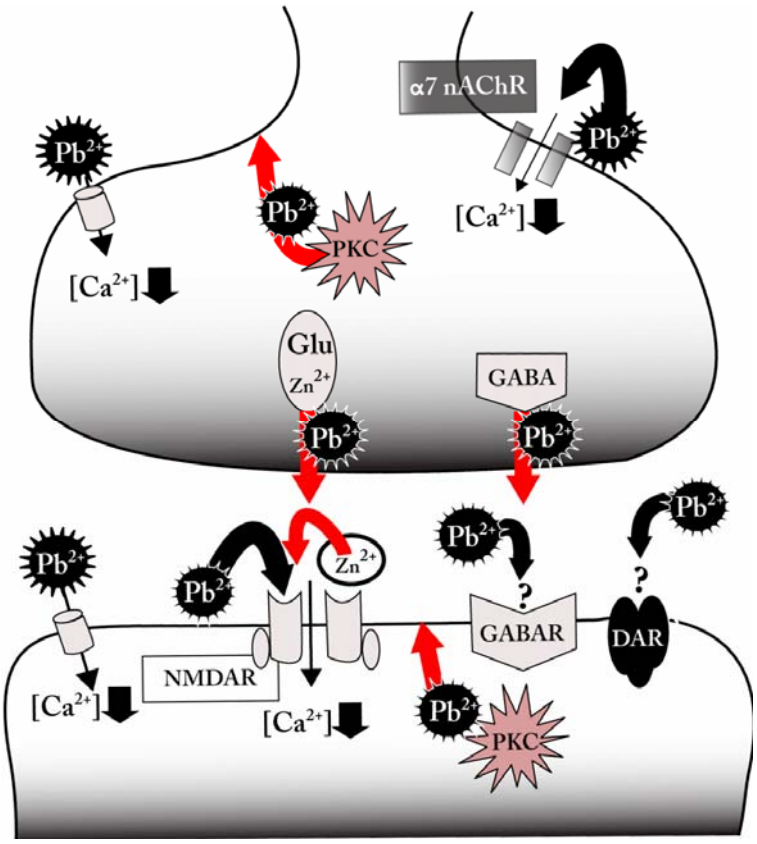

Figure 2-1. Multiple possible molecular targets for interference by Pb^{2+} ion at nerve synapses.

Source: Nihei and Guilarte (2002).

presynaptic terminal include synaptic vesicles, ionotropic receptors, Ca^{2+} and other channel proteins, and kinase proteins. At the postsynaptic interface, ionotropic proteins, dopamine receptors, protein kinase-C isoenzymes and ion channel proteins are amongst the proteins subject to interference by Pb^{2+} (Nihei and Guliarte, 2002).

Additional information concerning the physical aspects of Pb coordination chemistry and its role in biological systems can be obtained from the substantial review by Claudio et al. (2003). An extensive discussion of the neuro- and other toxic effects associated with exposure to Pb can be found in Chapter 5 of this document.

2.2 SOURCES OF LEAD

In this section, information is summarized with regard to a number of major sources of Pb, categorized as natural sources, stationary point sources, and mobile sources. In addition to these categories, fugitive emissions such as resuspension of Pb in soil and dust can be important. Resuspension is considered a transport route and is discussed in Section 2.3.

2.2.1 Natural Sources

Common natural sources of airborne Pb include volcanoes, sea-salt spray, biogenic sources, wild forest fires, and wind-borne soil particles in rural areas with background soil concentrations. Natural sources combined contribute an estimated 19,000 metric tons of Pb to the air each year (Nriagu and Pacyna, 1988). However, there is significant variability in the Pb emissions from volcanoes and forest fires and considerable uncertainty in biogenic and sea-salt emissions of Pb (Nriagu, 1989). Table 2-6 shows the median value and the range of annual emissions worldwide for natural sources of airborne Pb.

Table 2-6. Worldwide Annual Emissions of Lead from Natural Sources

Source	Amount Emitted: Range (thousands of metric tons/yr)	Amount Emitted: Median (thousands of metric tons/yr)
Wind-borne soil particles	0.3-7.5	3.9
Sea salt Spray	0.02-2.8	1.4
Volcanoes	0.54-6.0	3.3
Wild Forest Fires	0.06-3.8	1.9
Biogenic, continental particulates	0.02-2.5	1.3
Biogenic, continental volatiles	0.01-0.038	0.20
Biogenic marine sources	0.02-0.45	0.24
Total	0.97-23	12

Source: Nriagu (1989).

Natural Pb emissions worldwide are somewhat greater than the estimated 1600 metric tons/year of Pb emitted from anthropogenic stationary and mobile sources in the United States in the year 2002 (U.S. Environmental Protection Agency, 2003). However, many countries around the world have much greater Pb emissions than the United States from stationary and mobile sources, including several countries that still use leaded gasoline. Furthermore, the EPA estimate does not account for emissions of Pb in resuspended soil. Harris and Davidson (2005) estimate that stationary and mobile source emissions account for only about 10% of the total Pb emissions in the South Coast Air Basin of California; the remaining 90% of the emissions are from resuspended soil. The soil contains elevated Pb levels because of the many decades of leaded gasoline use. Therefore, on a worldwide basis, anthropogenic emissions of Pb are expected to be much greater than natural emissions.

There are four stable isotopes of Pb: ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb. The last three of these isotopes are produced by decay of ^{238}U, ^{235}U, and ^{232}Th respectively. The concentrations of natural vs. anthropogenically derived Pb in environmental media are often determined through isotopic ratios. Most minable Pb ores exhibit ratios of ^{206}Pb/^{207}Pb between 0.92 and 1.20 (Erel et al., 1997). Rock released or "natural" Pb, however, generally exhibits a higher ^{206}Pb/^{207}Pb ratio.

Deep soil samples converge to ratios of ^{206}Pb/^{207}Pb ~ 1.21 and ^{208}Pb/^{206}Pb ~ 2.05, which are considerably different from the natural ratios found in adjacent bedrock (Erel et al., 1997). For more information on isotopic ratios of Pb and their uses as environmental tracers, see Chapter 7 of this document.

Natural aerosol Pb tends to have large particle sizes (Reuer and Weiss, 2002). As a result, it deposits rapidly and has an atmospheric residence time of a few hours to ~10 days (Reuer and Weiss, 2002). The average downward flux is estimated as 0.012 mg m^{-2} yr^{-1} for natural Pb in all forms (Bindler et al., 1999).

Lead concentrations in air and soil have most likely been elevated by anthropogenic activities at least since the rise of the Greek and Roman societies, both of which extensively used Pb. The natural background (i.e., pre-Greek and Roman eras) concentration of Pb in soil is <0.1 ppm (Bindler et al., 1999; Erel et al., 1997). This is significantly higher than the adjacent bedrock but is approximately equal to concentrations found in bedrock residues such as quartz and clay (Erel et al., 1997). An estimated 3.1×10^{14} metric tons of Pb are dispersed within the

continental crust (Reuer and Weiss, 2002). Of this, ~9.3×10^7 metric tons of Pb are found in Pb ores. Table 2-7 lists the naturally occurring concentrations of Pb in bedrocks, ocean crusts, and continental crusts. Spatially, background levels of Pb vary considerably.

Table 2-7. Naturally Occurring Lead Concentrations in Major Rock Types

Lithology	Natural Pb Concentration (ppm)
Continental Crust	15.0
Oceanic Crust	0.9
Basalts, Gabbros	3.5
Limestones	5.0
Granulites	9.8
Greywackes	14.0
Gneisses, Mica Schists	22.0
Shales	22.0
Granites	32.0

Source: Reuer and Weiss (2002).

Natural Pb in surface water is derived from four different sources: biogenic material, aeolian particles, fluvial particles, and erosion (Ritson et al., 1994). As discussed in Chapter 7 (Section 7.2.2), Pb is generally present in aquatic systems as Pb salts, such as $PbSO_4$, $PbCl_4$, $Pb(OH)_2$, etc. About 90% of natural Pb in the open ocean is in the dissolved phase (Reuer and Weiss, 2002). Organic ligands are complexed with 50 to 70% of this Pb, with the balance of Pb in open ocean waters being found in inorganic compounds (Reuer and Weiss, 2002). Biological particles in the open ocean scavenge a significant portion of the Pb complexes, which have an estimated two-year residence time in the surface waters (Reuer and Weiss, 2002).

A naturally occurring radioactive isotope of Pb, ^{210}Pb, is commonly used as a tracer to determine how particles are transported through the environment. The source of ^{210}Pb is the ^{238}U decay series. In this process, gaseous ^{222}Rn is produced, which escapes from the soil and enters the atmosphere. As radon decays into ^{210}Pb, the particulate Pb deposits onto soils and

surface waters all over the world. The surfaces of all soils have been exposed to atmospherically derived ^{210}Pb particles (Kaste et al., 2003).

Particles of ^{210}Pb tend to be submicron, with an average size of 0.53 μm AMD (Winkler et al., 1998). The mean residence time for ^{210}Pb in the air is ~4 to 5 days but has been estimated to be as long as 8 days with some seasonal variability (Winkler et al., 1998). The downward flux has been estimated as 136 Bq m^{-2} yr^{-1} for ^{210}Pb (Joshi et al., 1991).

Atmospheric deposition is likely the largest source of ^{210}Pb to water bodies. Leaching of Pb naturally contained in host rock is a very small source to water (Toner et al., 2003). In surface waters, ^{210}Pb is primarily in particulate form, whereas dissolved Pb is transported more readily (Joshi et al., 1991). Dissolved ^{210}Pb is scavenged by suspended matter (Carvalho, 1997). The residence time of dissolved ^{210}Pb is ~30 days, although partial re-dissolution from bottom sediments probably occurs (Carvalho, 1997). One estimate found that ~56% of atmospherically derived ^{210}Pb in lakes of the Canadian Shield was retained in the sediment (Joshi et al., 1991).

Many authors have measured concentrations of ^{210}Pb in plants (including foodstuffs) and animals (including humans). Holtzman (1978) summarized these measurements. Concentrations in United States vegetation range between 30 pCi/kg and 70,000 pCi/kg for wheat and lichens respectively. The estimated human consumption of ^{210}Pb from vegetation averages 1.4 pCi/day in the United States. Overall, the concentrations of ^{210}Pb in animals vary significantly depending on the type of tissue or organ measured. However, concentrations are generally higher in animals with higher rates of Pb intake.

2.2.2 Lead Emissions in the United States

Currently, the major use of Pb in the United States is in Pb-acid batteries, for which the demand is increasing (Socolow and Thomas, 1997). Other major uses are for glass, paints, pigments, and ammunition. United States consumption of Pb by industry is shown in Figure 2-2. The consumption reached ~1.4 million metric tons per year in the mid 1990s (Socolow and Thomas, 1997). Approximately 910,000 metric tons of this was secondary production, indicating high rates of Pb recycling.

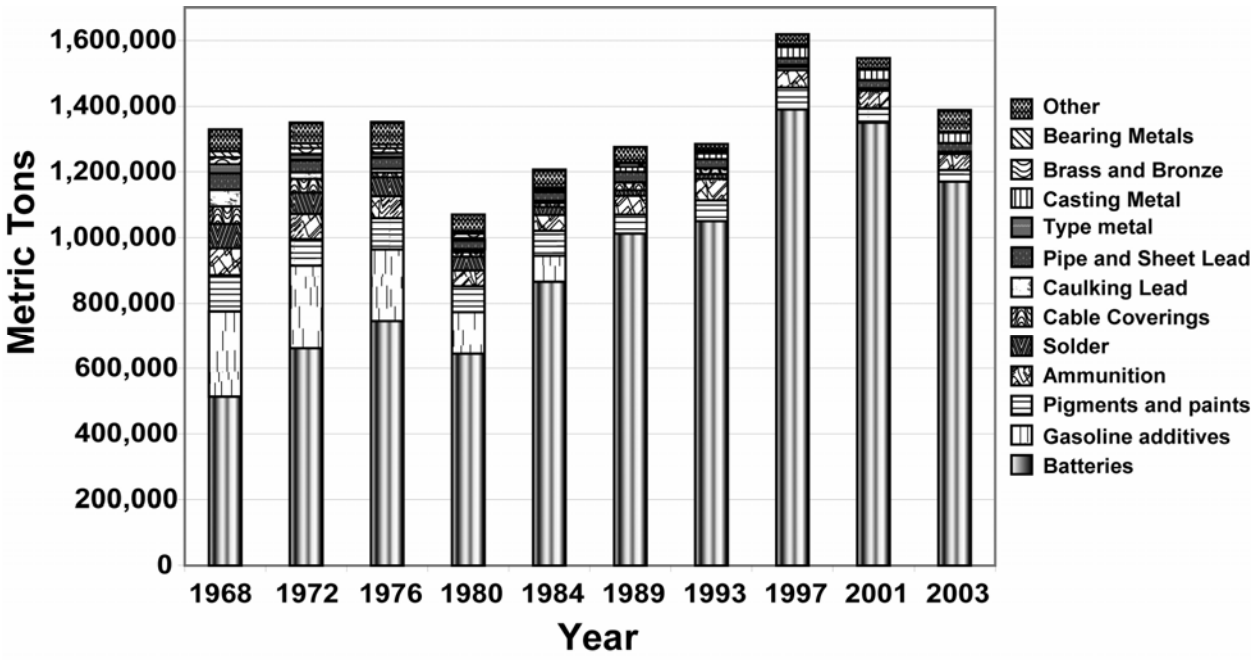

Figure 2-2. Annual lead production and use in the United States (1968 - 2003).

Source: U.S. Bureau of Mines (1968-1995) and USGS (1996-2003).

Table 2-8 presents estimates of annual emissions rates for Pb in the U.S. from 1990 and 2002 (20 tons per year or more in 2002). These values were extracted from the 1990 Emissions Inventory of Forty Potential Section 112(k) Pollutants and the 2002 National Emissions Inventory (NEI) (U.S. Environmental Protection Agency, 1999, 2006a). For criteria air pollutants, sources are required to report emissions either annually or every three years, depending on the size category of the sources. The NEI contains estimates of facility-specific air pollutant emissions and the source-specific factors necessary for modeling such as location and facility characteristics (e.g., stack height, exit velocity, temperature). Complete source category coverage is needed, and the NEI contains estimates of emissions from stationary point and nonpoint (sources such as residential heating that are inventoried at the county level) and mobile source categories. The NEI contains individual stack and fugitive emissions estimates at individual geocoordinates for point sources. County level estimates are provided in the NEI for nonpoint and mobile sources. Further information on the NEI is available at: http://www.epa.gov/ttn/chief/net/index.html. Note that all emission rates are estimated based on periodic reporting by sources of their activities, production, and fuel consumption, multiplied

Table 2-8. Annual Air Emission Rates for U.S. Lead Sources (20 Tons per Year or Greater) for 1990 and 2002, Ordered by Emissions Levels in 2002. These Data were Extracted from the 1990 Emissions Inventory of Forty Potential Section 112(k) Pollutants and the 2002 National Emissions Inventory (NEI) (U.S. EPA. 2006a).

Source Category Name	1990 112(k) Total Emissions (TPY) [a]	2002 Total Emissions (TPY)[a]	1990% emissions	2002% emissions
ALL CATEGORIES	**3270.0**	**1435.0**		
Industrial/Commercial/ Institutional Boilers & Process Heaters	33.7	247.0	1.03	17.21
Utility Boilers: Coal	72.0	165.4	2.20	11.53
Mobile sources	1198.0	142.8	36.64	9.95
Iron and Steel Foundries	13.5	110.0	0.41	7.67
Hazardous Waste Incineration (incl onsite)	96.7	69.7	2.96	4.86
Primary Pb Smelting	220.0	58.9	6.73	4.10
Electric Services	c	53.2	—	3.71
National Security	d	34.1	—	2.38
Municipal Waste Combustors: Small & Large	80.4	32.8	2.46	2.29
Integrated Iron & Steel Manufacturing (b)	23.5	32.7	0.72	2.28
Pressed and Blown Glass and Glassware Manufacturing	52.4	30.4	1.60	2.12
Secondary Nonferrous Metals	55.8	28.3	1.71	1.97
Pb and Zinc Ores	c	25.6	—	1.78
Pb Acid Battery Manufacturing	54.8	24.9	1.68	1.73
Stainless and Nonstainless Steel Manufacturing (EAF)	84.0	23.4	2.57	1.63
Primary Copper Smelting	152.0	21.7	4.65	1.51
Portland Cement Manufacturing	e	21.4	—	1.49
Primary Metal Products Manufacturing	2.0	20.5	0.06	1.43

a) Categories with emissions >20 TPY in 1990 112(k) or 2002 NEI.
b) Listed as Blast Furnaces and Steel Mills in 1990 112(k).
c) Source category was not included in the 1990 112(k) inventory.
d) Source category was included in the 1990 112(k) inventory but no Pb emissions were identified.
e) Source category was included in 1990 112(k) inventory but no Pb emissions were quantified.

by sector-wide average emission factors per unit of activity, production, or fuel consumed. There are numerous uncertainties in the estimation of emissions inventory data. Emissions measurements on a per facility basis are scarce. EPA's AP-42 document includes emission factors for many different processes and operations The uncertainties underlying emissions estimates are greater for some sources than others, and the AP-42 document includes emission factor ratings from A (excellent) through E (poor) based on the quality of the testing or measurement data and how well the factor represents the emission source. Further information on emission factors is available at: http://www.epa.gov/ttn/chief/ap42/.

Regardless of these uncertainties, the long-term trend in U.S. Pb emissions is very clear. Total Pb emissions to the air declined by about half between 1990 and 2002, from ~3300 tons per year to ~1400 tons per year, largely due to the decline in emissions from mobile sources. In the summary data for 1990, mobile sources were by far the largest source of Pb emissions to the air. In 2002 NEI data, mobile sources remain an important source of airborne Pb emissions (from specific types of mobile sources discussed further in Section 2.2.4), but industrial sources and combustion sources have become the major sources of U.S. Pb emissions. As shown in Table 2-8, the largest U.S. emissions sources for airborne Pb in 2002 are industrial sources, such as industrial/commercial/institutional boilers or process heaters and utility boilers.

Another source of information on U.S. Pb emissions to air is the EPA's Toxics Release Inventory (TRI) (U.S. Environmental Protection Agency, 2006b). Reported emissions to the air in 2004, including fugitive and point source emissions from U.S. facilities, totaled over 1 million pounds, or ~500 tons. This is consistent with the emissions estimates reported for 2002 in the NEI (1400 tons), in that the TRI includes emissions data reported by facilities, whereas the NEI includes estimated emissions from a broader group of sources. For more information about the TRI, see: http://www.epa.gov/ebtpages/emerreportingtoxicsreleaseinventorytri.html.

Information is also available from the peer-reviewed literature from studies done at specific sites. These data are summarized in the following discussions. Section 2.2.3 focuses on data available for numerous stationary source categories, and Section 2.2.4 presents data on Pb emissions from mobile sources.

2.2.3 Stationary Sources

As observed in the preceding section, emissions estimates and measurements on a per facility basis are scarce. The AP-42 document of the EPA includes emission factors for many different processes and operations. For Pb-processing facilities, these emission factors are usually expressed as grams of Pb emitted per kg of Pb processed (U.S. Environmental Protection Agency, 2005). In general, AP-42 data are not listed in the following sections except in the absence of newer, more robust or peer-reviewed data on process emissions. Although AP-42 emission factors can provide a first order estimate, they are limited in that they are often derived from one individual source and do not reflect the variability between sources (U.S. Environmental Protection Agency, 2006c). Also, in many cases, AP-42 emission factors do not account for process parameters. In some cases, AP-42 may complement the data listed below, and the reader is referred there for emission factors not given in this chapter. Up-to-date, accurate emissions estimates are critical as inputs to models predicting airborne concentrations, and more research in this area is needed.

Primary and Secondary Lead Smelters

Primary Pb smelting is the process by which elemental Pb is recovered from Pb ore. Lead ore is primarily in the form of galena (PbS) but can also occur as plattnerite (PbO_2), cerussite ($PbCO_3$), and anglesite ($PbSO_4$) (Reuer and Weiss, 2002). Producing elemental Pb from ore involves three processes – sintering, reduction, and refining – each with its own characteristic emissions. Primary Pb production in the United States emitted about 58.9 metric tons of Pb in 2002, ~4% of total anthropogenic Pb emissions in the United States (U.S. Environmental Protection Agency, 2006a). Currently, there is only one remaining primary Pb smelting facility still operating in the United States. The Pb emissions from this facility, Doe Run's Herculaneum, Mo. facility, were 25 tons in 2005.

Secondary Pb smelters reclaim scrap Pb. Both the principal input to and the principal major product market of secondary smelters are Pb-acid batteries. Secondary Pb production contributed 82% of total Pb production in 2003 (USGS, 2003). Secondary Pb production in the United States emitted about 4.3 metric tons of Pb in 2002, <1.0% of total anthropogenic Pb emissions in the United States (U.S. Environmental Protection Agency, 2006a). Although recycling of Pb-acid batteries with minimal emissions may be possible (Socolow and Thomas,

1997) secondary smelters and battery recycling facilities are still one of the most significant stationary sources of airborne Pb emissions.

The quantity of Pb emitted from a given facility is highly variable and depends on facility processes and meteorological conditions such as wind speed and ambient temperature. Emissions estimates are typically performed through direct measurements, mass balances, process models, inverse inferences, or emissions factors (Frey and Small, 2003).

Emissions from smelters have been measured in several cases. A survey of approximately 50 European Pb smelters had mean emission factors of 0.1 grams and 0.05 grams of Pb emitted per kg of Pb processed for primary and secondary Pb smelters respectively (Baldasano et al., 1997). Measurements of emissions from the blast furnace of a primary smelter were between 1.2 and 3.8 kg Pb/hr (Bennett and Knapp, 1989). The acid-sinter at the same plant emitted between 0.4 and 8.5 kg Pb/hr (Bennett and Knapp, 1989). Emissions occur during every stage of the overall smelting process. Because the process emissions mostly are controlled to conserve raw materials, the largest source of emissions is likely to be fugitive dust from the transport, grinding, and storage of battery scrap (Kimbrough and Suffet, 1995).

Much work has been done to determine the species of Pb emitted from the various smelting processes. The fraction of Pb in particulate matter (PM) emissions varies significantly between processes and depends on the type of furnace used. However, Pb is often the dominant element in smelter emissions. Lead can be emitted either in PM or in fumes. Lead fume emissions are particularly high if Pb blast furnace bullion is transferred in an open ladle (Wang and Morris, 1995). Major components of particulate Pb emissions are PbS, $PbSO_4$, $PbSO_4$•PbO, and elemental Pb; and minor species are $PbCO_3$, PbO, Pb silicates, and PbO litharge (Batonneau et al., 2004; Harrison and Williams, 1983; Ohmsen, 2001; Sobanska et al., 1999; Rieuwarts and Farago, 1995).

The distribution of particle sizes varies depending on temperature, process, and the conditions of each facility. Ohmsen (2001) found that Pb emissions from a blast furnace tend to be less than 1 μm in size and have a smaller diameter than particulate emissions from either the sintering process or storage areas. Higher temperatures (>600 °C) in the blast furnace tend to produce emissions with finer particle sizes. Dusts from the raw materials area tend to fall between 10 and 100 μm, whereas dusts from the refinery tend to fall between 1 and 30 μm (Ohmsen, 2001). Sobanska et al. (1999) found that just 15% of dust particles by mass emitted

from a "water jacket" furnace were <10 µm in diameter, and the remaining 85% fell between 10 and 100 µm. Measurements of Harrison et al. (1981) at a primary smelter found particles derived from combustion processes to be typically between 0.1 and 2 µm, but also showed that these particles could agglomerate to more than 10 µm if confined to ventilation ducts. Reported sizes from primary smelting processes are shown in Table 2-9.

Table 2-9. Mass-Median Aerodynamic Diameters for Particles from Various Processes at Primary Lead Smelters

	Average Particle Size		
Primary Smelter Process	**Harrison et al. (1981)**	**Ohmsen (2001)**	**Bennett and Knapp (1989)**
Raw Materials	—	40 µm (range = 10-100 µm)	—
Sinter	5.1 µm	range = 10-300 µm	0.91 µm, 80% of particles <10 µm
Blast Furnace	3.4 µm	90% of particles were <1 µm	1.1 µm, 88% of particles <10 µm
Copper Drosser	9.4 µm	range = 10-300 µm	—
Refinery	—	range = ~1-100 µm, mostly <20 µm	—

Note: Where there were multiple data points, geometric means were used. Data for Harrison et al. (1981) were occasionally given as >11 µm. These values were replaced with 11 µm before calculating the geometric mean. Thus, these values represent a lower limit.

Source: Harrison et al. (1981), Ohmsen (2001), Bennett and Knapp (1989).

Lead concentrations in stack outlets have been measured in several cases. Measurements taken at the stack of a blast furnace in a primary smelter ranged between 3.7 and 7.3 mg/m^3 (Bennett and Knapp, 1989). Stack concentrations at the sinter plant of the same facility ranged between 4.5 and 71.0 mg/m^3 (Bennett and Knapp, 1989). Two stacks on a blast furnace at a secondary smelting facility had Pb concentrations of 0.002 and 0.0137 mg/m^3 (Sturges and Harrison, 1986). The average values of ~50 European smelters were 2 mg/m^3 for both primary and secondary smelters (Baldasano, et al., 1997).

The ambient air concentrations in the immediate vicinity of smelters tend to be elevated to varying degrees depending on facility operations and meteorological conditions. In the UK, an

increase of 1.5 μg/m^3 in the local ambient air was attributed to the emissions of a single secondary Pb smelter (Sturges and Harrison, 1986). Fenceline measurements at two secondary smelters located in California ranged between 0.85 and 4.0 μg/m^3 (Kimbrough and Suffet, 1995). Air-Pb concentration data measured at 50 m, 500 m, and 800 m from the plant were slightly lower but generally on the same order of magnitude as the fenceline values. Ambient air concentrations measured at 12 sites within several hundred meters of three secondary Pb smelters in Manitoba were elevated (Tsai, 1987). The geometric means of these samples, which were taken over three month time spans, ranged between 0.11 and 1.69 μg/m^3. Also, the area was shown to be much less likely to meet the Manitoba guideline of <5 μg/m^3 for a 24-hour average when the smelters were operating than when they were not.

Nonlead Metallurgical Processes

Emissions of Pb from nonlead smelters can be significant. Emissions from smelters, metal works, and metal refineries depend on the type of equipment used to process the metals, the concentrations of Pb in the initial material (ore, recycled material, or alloy), the type and effectiveness of pollution controls at the facility, and the temperature of operations (Pacyna, 1986). Little work has been done to speciate Pb emissions from metallurgical facilities, although Pb emissions from a primary copper-nickel smelter are primarily in the form of PbO (Barcan, 2002). Emissions from non-Pb metallurgical processes are summarized in Table 2-10.

Ore Mining and Processing

Lead mining occurs in 47 countries, although primary Pb production is on the decline (Dudka and Adriano, 1997). World mine production of Pb is ~2.8 million metric tons per year (Wernick and Themelis, 1998). The reserve base of Pb is estimated to be about 120 million metric tons, which will sustain current rates of mine production for 43 years (Wernick and Themelis, 1998).

Mines can be a significant source of metal emissions to the atmosphere. Lead and zinc ores, which are often mined together, frequently contain high concentrations of cadmium and arsenic (Pacyna, 1986). An emission factor for Pb mines has been reported as 0.91 grams of Pb emitted to the air per kg of Pb mined (Pacyna, 1986).

Table 2-10. Emissions of Lead from Nonlead Metallurgical Processes

Metallurgical Plant	Lead Emissions	Particle Sizes MMAD = Mass median aerodynamic diameter	Location	Source
Aluminum (secondary)	0.81±0.014% of PM emissions	Fine (<2.5 µm)	Philadelphia, USA	Olmez et al. (1988)
Aluminum (secondary)	0.098±0.031% of PM emissions	Coarse (2.5-10 µm)	Philadelphia, USA	Olmez et al. (1988)
	1.01×10-3-3.52×10-3 kg/mt produced (venturi scrubber)			
	3.38×10-6-7.40×10-6 kg/mt produced (baghouse)			
Aluminum (secondary) – burning drying	1.05×10-2-1.13×10-2 kg/mt produced (multiple cyclones)	n.a.	U.S.	U.S. EPA (1998)
Aluminum (secondary) – reverberatory furnace	5.0×10^{-4}-1.1×10^{-3} kg/mt processed (baghouse)	n.a.	U.S.	U.S. EPA (1998)
Antimony	0.17±0.04% of PM emissions	Fine (<2.5 µm)	Philadelphia, USA	Olmez et al. (1988)
Antimony	0.11±0.02% of PM emissions	Coarse (2.5-10 µm)	Philadelphia, USA	Olmez et al. (1988)
Brass/Bronze refinery	0.01-1% of PM emissions	n.a.	n.a.	Lee and Von Lehmden (1973)
Brass/Bronze refinery – blast furnace	16 g/ton produced	n.a.	n.a.	Pacyna (1986)
Brass/Bronze refinery – crucible furnace	10 g/ton produced	n.a.	n.a.	Pacyna (1986)
Brass/Bronze refinery – cupola furnace	65 g/ton produced	n.a.	n.a.	Pacyna (1986)
Brass/Bronze refinery - reverberatory furnace	60 g/ton produced	n.a.	n.a.	Pacyna (1986)
Brass/Bronze refinery – rotary furnace	60 g/ton produced	n.a.	n.a.	Pacyna (1986)

Table 2-10 (cont'd). Emissions of Lead from Nonlead Metallurgical Processes

Metallurgical Plant	Lead Emissions	Particle Sizes MMAD = Mass median aerodynamic diameter	Location	Source
	25 kg/mt produced (high-leaded alloys)			
	6.6 kg/mt produced (red and yellow Pb alloys)			
Brass/Bronze production	2.5 kg/mt produced (other alloys)	n.a.	U.S.	U.S. EPA (1998)
Copper-Nickel	184 mt/yr, 21 kg/hr	1.2 µm MMAD	Copper Cliff, Ontario	Chan and Lusis (1986)
Copper-Nickel	13.4 mt/year	0.9 µm MMAD	Falconbridge, Ontario	Chan and Lusis (1986)
Copper-Nickel (primary)	0.6-1.4% of PM emissions	n.a.	Monchegorsk, Russia	Barcan (2002)
Copper-Nickel (primary)	2.3-3.6 kg/ton produced	n.a.	Poland	Pacyna (1986)
Copper-Nickel (primary)	3.1 kg/ton produced	n.a.	n.a.	Pacyna (1986)
Copper (primary) smelter	3.0×10^{-2} kg/ton produced	n.a.	U.S.	U.S. EPA (1998)
Copper (primary) converter	0.27 kg/ton produced	n.a.	U.S.	U.S. EPA (1998)
Copper (secondary) reverberatory furnace	2.5 – 25 kg/mt produced	n.a.	U.S.	U.S. EPA (1998)
Copper (secondary) smelter	5.0×10^{-4} kg/mt processed	n.a.	U.S.	U.S. EPA (1998)
Copper Smelter - furnace	0.24-0.52 kg/hr	0.87 µm MMAD	n.a.	Bennett and Knapp (1989)
Copper Smelter - sinter	below detection	<0.10 µm MMAD	n.a.	Bennett and Knapp (1989)
Copper Smelter (secondary)	54-214 g/ton produced	n.a.	n.a.	Pacyna (1986)
Iron Ore Recovery and Ni refinery	6 mt/year	Coarse (2.5-10 µm)	Copper Cliff, Ontario	Chan and Lusis (1986)

Table 2-10 (cont'd). Emissions of Lead from Nonlead Metallurgical Processes

Metallurgical Plant	Lead Emissions	Particle Sizes MMAD = Mass median aerodynamic diameter	Location	Source
	0.05-1.10 kg/mt produced (no control device) 7.80×10^{-4} kg/mt processed (afterburner, venturi scrubber)			
Iron foundry cupola	6.95×10-4-2.23×10-3 kg/mt produced (baghouse)	n.a.	U.S.	U.S. EPA (1998)
Iron foundry –reverberatory furnace	6.00×10^{-3}-7.00×10^{-2}kg/mt produced (no control device)	n.a.	U.S.	U.S. EPA (1998)
Iron foundry – electric induction furnace	4.45×10^{-3}-5.00×10^{-2} kg/mt produced (no control device)	n.a.	U.S.	U.S. EPA (1998)
Iron foundry – casting	2.40×10^{-3} kg/mt processed (afterburner, venturi scrubber)	n.a.	U.S.	U.S. EPA (1998)
Iron and Steel foundry	0.01-0.1% of PM emissions	n.a.	n.a.	Lee and Von Lehmden (1973)
Steel works - electric-arc furnace	4.1-16.3 g/ton produced	n.a.	n.a.	Pacyna (1986)
Zinc-Cadmium (primary)	1.2-25 kg/ton produced	n.a.	n.a.	Pacyna (1986)
Zinc Smelter - furnace	0.86-1.5 kg/hr	1.8-2.2 µm MMAD	n.a.	Bennett and Knapp (1989)
Zinc Smelter - sinter	3.6-6.0 kg/hr	0.9-2.1 µm MMAD	n.a.	Bennett and Knapp (1989)

Source: Olmez et al. (1988), Lee and Von Lehmden (1973), Pacyna (1986), Chan and Lusis (1986), Barcan (2002), Bennett and Knapp (1989).

Since Pb is mined in the form of galena (PbS), emissions from Pb mines tend also to be in the form of galena (Dudka and Adriano, 1997). However, other species have been detected. In mine spoils, Pb is typically galena and secondary alternation products such as plumbojarosite [$PbFe_6(SO_4)_4(OH)_{12}$] (Rieuwerts and Farago, 1995). Other Pb forms detected in the vicinity of mines are: pyromorphite [$Pb_5(PO_4)_3Cl$], which has a low bioavailability; $PbCO_3$, which is formed from the weathering of galena in the soil; leadhillite [$Pb_4SO_4(CO_3)_2(OH)_2$]; $PbS{\bullet}Bi_2S_3$; Pb oxides; Pb silicates; and $PbSO_4$ (Rieuwerts and Farago, 1995).

Although mining can be considered a point source to air, mine wastes can have a major widespread effect on soil and water (Riewerts and Farago, 1995). Mines produce four different types of large-volume waste: mine waste, which consists of overburden and barren rocks, tailings, dump heap leachate, and mine water (Dudka and Adriano, 1997). Tailings especially are major sources of metal contamination to soil and water (Bridge, 2004). Acid mine drainage can contain highly elevated levels of Pb, >3000 μg/L, and can contaminate vast areas (Bridge, 2004; Kurkjian et al., 2004). Soil contamination from both active and abandoned mines can be a significant source of airborne Pb from fugitive or wind blown matter. Resuspension of contaminated soil is addressed later in this chapter, and soil Pb concentrations near mines are discussed in Chapter 3.

Mining of materials other than Pb can also release Pb to the atmosphere. Zinc-copper ores, for example, contain Pb in the range of 100-100,000 ppm (Lee and Von Lehmden, 1973), and about 6.1% of all Pb in the United States is extracted from "zinc mines" (Dudka and Adriano, 1997).

In an underground gold mine, high Pb-particulate concentrations were associated with blasting (Annegarn et al., 1988). These particles were primarily Pb oxides and submicron in size. A source apportionment analysis on airborne PM in an underground gold mine found that the significant sources of Pb were rock dust and diesel exhaust (McDonald et al., 2003). Concentrations of airborne Pb inside the mine were measured at 0.21 $\mu g/m^3$.

Stationary External Combustion: Coal Combustion

Coal combustion can be a significant local source of Pb emissions as well as a considerable regional source of airborne Pb. Coal is commonly burned as a fuel for utilities, industries, and commercial and institutional facilities. Coal utility boilers accounted for 165 tons

of Pb emissions in 2002 (10% of total emissions) (U.S. Environmental Protection Agency, 2006a). Coal is pulverized, fluidized, or gasified before combustion. Generally, Pb impurities will volatilize early in the combustion process, although the precise rate of vaporization depends on the distribution of Pb particles in the coal and the particle sizes (Lockwood and Yousif, 2000). As Pb vapors cool, they condense, either forming individual particles or condensing on the surface of ash particles (Lockwood and Yousif, 2000; Furimsky, 2000; Clarke, 1993; Pacyna, 1986). A high surface area to volume ratio makes fine ash particles better candidates for surface sorption than coarse particles. Additionally, recondensed Pb particles tend to be fine, with an average size of 0.2 μm (Lockwood and Yousif, 2000). The fine fraction of PM from coal combustion has an enrichment factor of ~22 (Lockwood and Yousif, 2000).

The primary contributor of Pb emissions from coal combustion is the Pb content of the coal itself. Lead is present in all coal samples in varying amounts, depending on the location of the coalfield and even the location of the coal sample within a coalfield. Generally, Pb is present in trace amounts in the form of PbS, but it can also be present as pyrite and PbSe (Lockwood and Yousif, 2000; Mukherjee and Srivastava, 2005). The type of the coal – either bituminous, subbituminous, or lignite – does not seem to correlate with the quantity of trace elements (Mukherjee and Srivastava, 2005). The age of the coal also does not seem to impact the Pb concentration (Ghosh et al., 1987). The most important factors contributing to Pb content of uncombusted coal seem to be local environmental conditions at the time the coal formed and relative proportions of organic and inorganic matter (Pacyna, 1986; Ghosh et al., 1987). Globally, Pb concentrations in coal range between 2 and 80 ppm (Mukherjee and Srivastava, 2005). Table 2-11 lists the range of Pb concentrations measured in four different coal components.

Table 2-11. The Range of Lead Concentrations in Coal Lithotypes

Coal Lithotype	Range of Lead Concentrations (ppm)
Vitrain	0.30 – 16.17
Clarain	4.84 – 17.55
Durain	4.10 – 11.76
Fusain	3.64 – 15.60

Source: Ghosh et al. (1987).

Coal is often combined with limestone to attenuate sulfur dioxide emissions. However, limestone can contain trace elements and has been shown to increase emissions of Pb by four to six times in a fluidized bed system compared to tests performed without a limestone addition (Clarke, 1993). Other measurements performed on a fluidized bed system found that increasing limestone increased particulate emissions of Pb, but decreased gaseous emissions of Pb. The overall emissions of Pb (gaseous + particulate) remained relatively constant (Furimsky, 2000). Limestone had a negligible effect on pressurized fluidized bed systems, although Pb emissions from gasification systems may increase with limestone additions (Clarke, 1993).

Emissions from coal combustion depend a great deal on the process conditions at a given facility. In addition to the type of boiler, conditions such as temperature, heating rate, exposure time at elevated temperatures, and whether the environment is oxidizing or reducing can affect emissions (Pacyna, 1986). For Pb, changes in the temperature affect the size of particles, the amount of Pb in the vaporized fraction, and the species of the emissions. At combustion temperatures of 1800 K, about 0.1% of the total ash produced was vaporized (Lockwood and Yousif, 2000). At 2800 K, the vaporized fraction of the ash was increased to 20%. The ratio of air to coal during combustion can also have a major effect on emissions (Furimsky, 2000). In a fluidized bed system, increasing the air to coal ratio from 1.0 to 1.10 decreased the gas to solid ratio for Pb emissions from 1.5 to 0.18 (Furimsky, 2000).

Uncontrolled combustion of coal can also occur – usually as natural, in-ground coal fires – and such combustion can emit Pb (Finkelman, 2004). Although such fires may, at times, be locally importance, they are not discussed in detail here.

Controlled combustion is the norm for industries and utilities. The major pollution control systems are electrostatic precipitators (ESP), wet scrubbers, and baghouses. In general, pollution control systems are most effective at removing large particles and are least effective at removing submicron particles. ESPs are highly efficient and can remove particles with >99.9% efficiency depending on particle size, ash resistivity, flue gas temperature, and moisture content (Clarke, 1993). ESPs are used at more than 90% of coal-fired utility boilers in the United States (Senior et al., 2000). Particles that escape EPSs typically range from 0.1 to 1.0 µm in diameter (Senior et al., 2000). Wet scrubbers are also more than 99% efficient, with the majority of particles that escape being <2 µm in size (Pacyna, 1986). Wet scrubbers are used less commonly than ESPs and baghouses (Senior et al., 2000). Baghouses or fabric filters are frequently used by

coal-fired utilities. As with ESPs and wet scrubbers, the collection efficiency of baghouses is a function of particle size (Senior et al., 2000). Baghouses are >99% effective with mass emissions averaging <20 mg/m^3 (Clarke, 1993).

Little published information is currently available regarding the actual quantity of Pb emitted from coal-fired boilers. The EPA AP-42 program publishes emission factors for typical coal-fired boilers, although using process data specific to a given facility is likely to be more accurate. Clarke (1993) reported emissions from fluidized beds. Of the processes tested, the Pb emissions were highest from a 0.5 m bed with a limestone sorbent, second highest with a 1.0 m bed without a limestone sorbent, and lowest with a 0.5 m bed without a limestone sorbent (Clarke, 1993). Reducing the depth of the fluidized bed by 50% decreased the emissions of trace elements by ~5 to 50%, probably because deeper beds undergo attrition of ash (Clarke, 1993). Olmez et al. (1988) reported on Pb mass fractions of PM in a stack of a coal-fired power plant. For fine particles, Pb constituted 0.04 ± 0.004%, whereas for coarse particles, Pb constituted 0.03 ± 0.002%. Coal combustion products that underwent long-range transport from the coal-fired power plants of the Midwest contributed an estimated 0.05 μg/m^3 to the ambient air in Boston (Thurston and Spengler, 1985). Table 2-12 lists the emission factors for three different types of coal, in three different types of power plants.

Table 2-12. Lead Emission Factors for Coal Combustion in Three Different Furnaces

Rank	Cyclone Furnace	Stoker Furnace	Pulverized Furnace	Source
Bituminous with control device	8.5×10^{-14} kg/J 2.10×10^{-4} kg/mt	128×10^{-13} kg/J	5.5×10^{-14} kg/J 2.10×10^{-4} kg/mt	Pacyna (1986) U.S. EPA (1998)
Bituminous without control device	2.18×10^{-13} kg/J	2.18×10^{-13} kg/J	2.18×10^{-13} kg/J	U.S. EPA (1998)
Subbituminous with control device	1.03×10^{-13} kg/J	1.56×10^{-13} kg/J	6.2×10^{-14} kg/J	Pacyna (1986)
Lignite with control device	1.44×10^{-14} kg/J	2.17×10^{-14} kg/J	92×10^{-13} kg/J	Pacyna (1986)
Pulverized coal	507 lb/10^{12} Btu			U.S. EPA (2005)
Anthracite		4.45×10^{-3} kg/mt		U.S. EPA (1998)

Source: Pacyna (1986), U.S. Environmental Protection Agency (1998, 2005).

The species of Pb emitted from coal depend on process conditions. $PbSO_4$ was found to be the dominant Pb compound in flue gas up to 1150 K (Lockwood and Yousif, 2000). Above this temperature, elemental Pb and PbO, both in the vapor phase, dominate. As the temperature increases, the equilibrium shifts toward elemental Pb (Lockwood and Yousif, 2000). In pulverized coal combustion at 1800K, the Pb species found in the gas phase were PbO, elemental Pb, PbCl, and $PbCl_2$ (Furimksy, 2000). The solid phase was comprised of PbO, $PbO{\bullet}SiO_2$, elemental Pb, and PbO_2 (Furimksy, 2000). As the flue gas cools, the Pb composition changes. $PbCl_2$ increases and is the main constituent of the gas phase before condensation occurs at 900K. If low rank low chlorine coal is used, then PbO and elemental Pb will dominate the gas phase. At 1500K, $PbSO_4$ dominates the particulate phase; at 1800K PbO_2 was the predominant Pb compound in the particulate phase (Furimksy, 2000).

Lead emissions from coal combustion in industrial, commercial, and residential boilers are similar to the values listed above for utility boilers. Table 2-13 lists emission factors for coal combustion.

Table 2-13. Lead Emissions from Industrial, Commercial, and Residential Coal Combustion

Coal-fired unit	Emission factor (g/metric ton)
Industrial cyclone boiler	1.2
Industrial stoker boiler	7.7
Industrial pulverized coal boiler	4.5
Commercial/Residential boiler (stoker or hand-fired)	2.7

Note: Data for industrial boilers, assuming 10% ash fraction and 85% efficient control devices.

Source: Pacyna (1986).

Stationary External Combustion: Fuel Oil Combustion

Fuel oil combustion constitutes 15% of fossil fuel energy production in the United States. (U.S. Environmental Protection Agency, 1998). As with coal, fuel oil is used to generate energy for utilities, industries, and commercial and residential boilers. The discussion below focuses on electric power utilities, which are the largest users of fuel oil.

Fuel oil is generally combusted in tangentially-fired or wall-fired boilers. Emissions of Pb from oil combustion depend on the process conditions, the amount of Pb in the oil, and the amount of sulfur in the oil (Pacyna, 1986) (see Table 2-14).

Table 2-14. Lead Emission Factors for Oil-Fired Utility Boilers

Boiler Type	Emission Factor	Control Device
Residual oil-fired boiler, No. 6 oil, normal firing	4.33×10^{-15} kg/J	None
Residual oil-fired boiler, No. 6 oil, normal firing	9.35×10^{-15} kg/J $5.43\times10^{-15} - 1.22\times10^{-14}$ kg/J	Flue gas recirculation
Residual oil-fired boiler, No. 6 oil, tangential firing	4.33×10^{-15} kg/J	None
Residual oil-fired boiler, No. 5 oil, normal firing	6.89×10^{-15} kg/J	None
Distillate oil grades 1 and 2	3.84×10^{-15} kg/J	None
Oil-fired utility boiler	2.6 lb/trillion Btu	PM control
Oil-fired utility boiler	9.0 lb/trillion Btu	PM/SO_2 control

Source: U.S. Environmental Protection Agency (1998).

The Pb concentration in the oil is the most important factor for determining eventual Pb emissions from combustion. Lead concentration in crude oil range between 0.001 to 0.31 ppm (Pacyna, 1986). The heavy fractions of crude oil tend to possess higher metal concentrations, trending to larger metal concentrations with increasing weight. Refining oil removes about 10% of metals (Pacyna, 1986).

As with coal, process conditions and the presence of pollution control devices greatly affect the rate and characteristics of emissions from fuel oil combustion. Emissions from oil-fired boilers depend on the efficiency of combustion and how much deposited material has built up in the boiler (Pacyna, 1986). Additionally, poor mixing, low flame temperatures, and a short residence time in the combustion zone cause overall particulate emissions to be greater and individual particle sizes to be larger (Pacyna, 1986). Oil, which is typically atomized prior to

combustion, will emit larger particles and have a higher particulate loading when atomization is done at low pressures. Conversely, high pressure atomization leads to smaller particles and lower particulate loadings (Pacyna, 1986). In general, about 90% of PM mass is <2.5 μm in diameter (Olmez et al., 1988).

Emission factors published in the literature are limited. An average emission factor for European oil-fired power plants was reported as 126 μg Pb/MJ for oil containing 1% sulfur (Pacyna, 1986). Lead emissions are higher for oils with greater sulfur contents. Olmez et al. (1988) report Pb mass fractions for two oil-fired power plants in Philadelphia. Lead was found to be 1.0% ± 0.2% and 1.8% ± 0.6% in the fine PM fraction in these two plants, respectively, and 0.48% ± 0.2% and 3% ± 0.4% in the coarse fraction. Lead in PM at the Philadelphia plants was enriched by more than a factor of 1000 compared to the Pb concentration in the fuel oil. Lead in PM for seven other oil-fired power plants was enriched by more than a factor of 100 (Olmez et al., 1988). A plant in Boston increased the ambient concentration of fine Pb aerosols by an estimated 0.05 $\mu g/m^3$ and the ambient concentration of coarse Pb aerosols by 0.003 $\mu g/m^3$ (Thurston and Spengler, 1985).

The combustion of used oil is also common. About 75% of used oil, which is generated in the transportation, construction, and industrial sectors, is burned as fuel oil (Boughton and Horvath, 2004). The Pb concentration of used oils is markedly higher than that of low-sulfur crude-based heavy fuel oils (Boughton and Horvath, 2004). Emissions from used oil combustion are estimated at ~30 mg of Pb from the combustion of 1 L of used oil. This is 50 to 100 times higher than emissions from crude-derived fuel oils.

Emission rates for industrial boilers are similar to those of utility boilers. Industrial oil-fired boilers are not usually equipped with pollution control devices. Approximately 6.4 g of Pb are emitted per 1000 L of fuel oil burned with a sulfur content of 1% (Pacyna, 1986). Commercial and residential boilers, which are also not typically equipped with pollution control devices, have emissions of ~3.3 g of Pb emitted per 1000 L of fuel oil (Pacyna, 1986).

Stationary External Combustion: Wood Combustion

Wood-fired boilers are used almost exclusively by industries that produce wood or wood products. These include pulp and paper mills, lumber production facilities, and furniture manufacturers (U.S. Environmental Protection Agency, 1998). The materials used as fuel may

include bark, slabs, logs, cuttings, shavings, pellets, and sawdust. During combustion, elemental pollutants such as Pb are converted to their oxide forms. These are hydrated and later carbonated under atmospheric conditions (Demirbas, 2003a).

As with coal and oil, the largest factor affecting emissions from wood combustion is the concentration of Pb in the fuel. Lead concentrations tend to be very low for virgin wood. The median Pb concentration in 24 pine and spruce samples was 0.069 ppm (Krook et al., 2004). The concentrations of Pb in spruce, beech, oak, pine, and ailanthus are listed in Table 2-15.

Table 2-15. Lead Concentrations in Biomass, Char, and Ash Samples from Spruce, Beech, Oak, Pine, and of Ailanthus Trees

Wood	Biomass (ppm)	Char (ppm)	Ash (ppm)
Spruce trunk wood	0.32[a]	2.5[a]	33.2[a,b]
Beech trunk wood	0.36[a]	2.6[a]	35.0[a,b]
Oak trunk wood	0.27[a]	2.1[a]	28.4[a,b]
Pine trunk wood	n.a.	n.a.	34.9[b]
Ailanthus trunk wood	n.a.	n.a.	32.7[b]
Spruce bark	0.38[a]	3.1[a]	5.2[a], 36.2[b]
Beech bark	0.43[a]	3.3[a]	3.8[a], 40.8[b]
Oak bark	0.31[a]	2.5[a]	4.0[a], 34.0[b]
Pine bark	n.a.	n.a.	38.7[b]
Ailanthus bark	n.a.	n.a.	35.7[b]

[a] Source: Demirbas (2003a).
[b] Source: Demirbas (2003b).

Waste wood recovered from construction and demolition sites is increasingly used as fuel. Although most of this wood is untreated, some can have elevated levels of metals from surface treatment of the wood or industrial preservatives (Krook et al., 2004). In addition, waste wood commonly contains contaminants such as metal pieces, concrete, stone, gravel, glass, and soil, which may increase metal emissions during combustion. Lead has been measured in waste wood at levels ~40 times higher than levels found in virgin wood. The median concentration of Pb in

recovered waste wood in Sweden was 33 ppm (Krook et al., 2004), whereas Pb in recovered waste wood from Germany and the Netherlands had a median value of 110 ppm.

Emissions of metals from wood are affected by process conditions. Good air-fuel mixing and high furnace temperatures keep emissions low (Demirbas, 2003a). Additionally, emissions depend on whether or not the wood was combined with other fuels, the feed rate, the physical state of the wood, the stack temperature, the geometry of the boiler, which can act as an inertial particulate collector, the draft setting, and the amount of moisture in the fuel (Demirbas, 2003a; Fels et al., 1990; Pacyna, 1986).

Pollution control devices may be present with large-scale wood-fired boilers. These can greatly reduce PM emissions. However, in a wood-burner installation in Ontario, a cyclone was found to have an efficiency of just 53% for total PM mass (Fels et al., 1990). For particles <2 μm in diameter, the PM concentrations downstream of the cyclone were actually greater than those upstream, probably indicating that larger particles were breaking apart during passage through the cyclone. The emissions of Pb from wood combustion are highly variable. The emission factor for wet fuel at a large-scale wood burner was 0.0006 g Pb/kg fuel (Fels et al., 1990). For dry fuel, emission factors were in the range <0.00035 to 0.0014 g Pb/kg fuel burned, with an average of 0.00056 g Pb/kg fuel (Fels et al., 1990). Emissions from a wood stove and a fireplace were estimated as 0.007 g Pb and 0.0047 g Pb per kg of wood burned, respectively (Pacyna, 1986). The recently updated AP-42 emission factor for Pb from wood residue combustion in boilers is 4.8×10-5 lb/MMBtu (U.S. Environmental Protection Agency, 2005).

Lead emissions from combustion of waste wood are higher than emissions from combustion of virgin wood. Although emission factors are not available, the concentration of Pb in ash from waste wood combustion is elevated above that from the combustion of virgin wood (Krook et al., 2004).

Data on particle sizes and species of emitted aerosols from wood combustion are not readily available.

Stationary Combustion Sources: Solid Waste Incineration

The amount of municipal waste incinerated (~15% of waste) has remained stable over the past decade. In earlier years, municipal waste incineration was an important source of Pb emissions; and, locally it is still a concern in some places (Walsh et al., 2001). In New York

City in the late 1960s, for example, Pb emissions from refuse incineration were between 602 and 827 tons per year, which was about 40 to 50% of Pb emissions from cars that totaled ~1752 metric tons in the same area (Walsh et al., 2001). Due to new requirements for the use of Maximum Achievable Control Technology (MACT), combustion is estimated to be <10 tons per year nationally.

Incinerator residue is partitioned into bottom ash, fly ash, and flue gas. Here the focus is on Pb in flue gas, due to its importance in increasing airborne Pb concentrations (Chang et al., 1999). Lead in incinerator effluents is derived primarily from the noncombustible materials that end up in refuse (Pacyna, 1986). Under MACT requirements, the most common air pollution control technology used at municipal waste incinerators is a spray dryer-fabric filter scrubbing system, enhanced with activated carbon injection.

Factors that affect the quantity of Pb emitted from incinerators include combustion temperature, the amount of Pb in the refuse, process conditions, moisture content, the addition of reactive species such as calcium, magnesium, and aluminum, and the addition of sorbents. Of all these factors, temperature seems to have the greatest impact on metal volatility (Chen and Yang, 1998). Metal volatilization is fast during the initial stages of combustion but levels off after about 15 minutes (Ho et al., 1993; Chen and Yang, 1998). When plastics only were burned, Pb volatility was at 18% at 600 °C, 61% at 800 °C, and 91% at 1000 °C (Chen and Yang, 1998). Figure 2-3 shows the percent volatility for Pb at four different combustion temperatures over 25 minutes of combustion time. Chang et al. (1999) derived the following relationship for Pb emissions from a fixed bed refuse incinerator in Taiwan:

$$\ln E(wt\%) = -3.083T^{1.257} + 3.659 \qquad (2\text{-}4)$$

where E is the weight percent of Pb in particulate emissions, and T is the combustion temperature in Kelvin.

The amount of Pb emitted is dependent on the quantity of Pb in refuse. Typical sources of Pb include paper, inks, cans and other metal scrap, and plastics. For U.S. municipal solid waste, Pb concentrations vary between 110 and 1500 ppm, with an average of about 330 ppm (Durlak et al., 1997). Because other countries have very different waste compositions, Pb concentrations elsewhere can vary greatly.

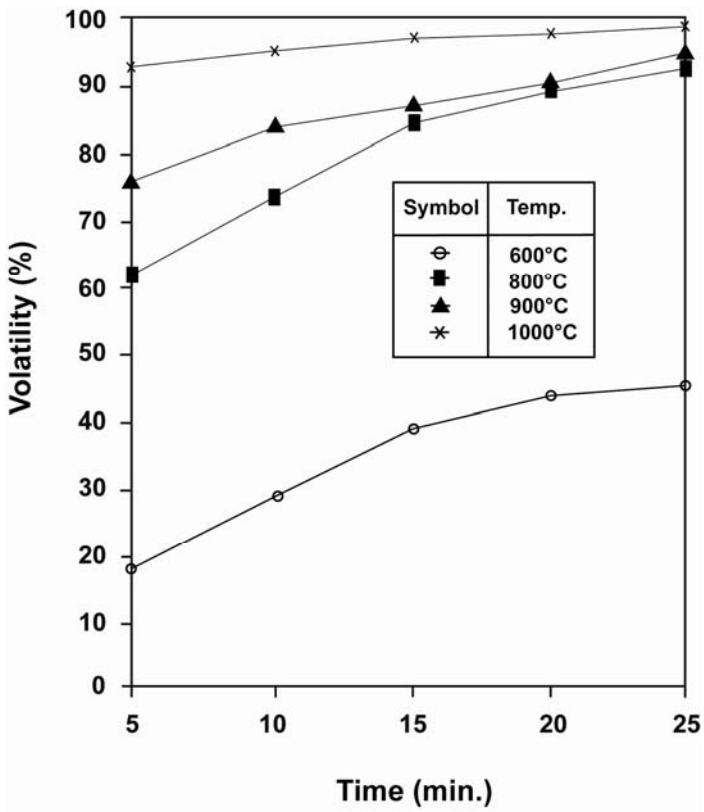

Figure 2-3. Percentage volatility of lead during combustion of plastics at four temperatures.

Source: Chen and Yang (1998).

Additionally, process conditions can affect Pb emissions. Increasing the amount of oxygen accelerates the rate of metal volatilization but does not seem to affect the overall amount of metal volatilized (Ho et al., 1993). Similarly, Chen and Yang (1998) observed that changing the N_2:O_2 ratio from 4:1 to 1:4 increased Pb volatility. An increase in the gas velocity can also increase Pb emissions, although this is a relatively minor effect (Chang et al., 1999; Chen and Yang, 1998).

The moisture content in an incinerator can affect the behavior of Pb. At a typical temperature of 950 °C, decreasing the moisture level from 37% to 5% increased Pb in the fly ash from 54% to 58% (Durlak et al., 1997). Similarly, decreasing the relative humidity from 60% to 40% at 900 °C increased the Pb volatility from 67% to 76%, respectively (Chen and Yang,

1998). In addition to these direct effects, moisture can indirectly affect emissions by altering the combustion temperature (Durlak et al., 1997).

Additives can reduce metal emissions from incinerators. Additives such as calcium, magnesium, and aluminum react with metals and bind them. This has been shown to reduce the formation of metal particles. Adding $Al(NO_3)_3$, for example, reduced quantities of $PbCl_2$ emitted (Ho et al., 1993). The addition of $Ca(OH)_2$ did not affect volatility at 600 °C (lower limit for combustion temperature) or at 1000 °C (upper limit for combustion temperature) (Chen and Yang, 1998). However, $Ca(OH)_2$ did appreciably limit Pb emissions at intermediate temperatures.

Sorbents can also reduce metal emissions. Sorbents function by binding metal vapors through heterogeneous chemical absorption and/or condensation before vaporized metals are able to form particles (Ho et al., 1993). In a fluidized bed incinerator, the efficiency of metal capture with sorbents varied between 4.9% and 94.5% (Ho et al., 1993), depending on temperature. Low efficiencies were observed at high and low temperatures, whereas optimal efficiency was observed in the intermediate range of ~600 to 800 C. Limestone was shown to be a more effective sorbent than sand.

Emissions from refuse incinerators have been reported as 0.018 g of Pb emitted per kg of refuse, assuming a control device with 85% efficiency (Pacyna, 1986). A source apportionment study showed that refuse incineration increased the ambient concentration of Pb by an estimated 0.008 $\mu g/m^3$ (Thurston and Spengler, 1985). This was observed after incinerators had been banned in the area, probably indicating prohibited residential refuse combustion. Lead in PM emissions has been reported to be between 6.9% and 8.9%, with an average of 8.1% (Pacyna, 1986). Three U.S. incinerators had emissions in which Pb constituted 8.2 ± 1.6% of the PM (Olmez et al., 1988).

Chlorine plays a critical role in determining the speciation of Pb emissions. Lead in the incineration system exists primarily as chlorine species (either PbCl or $PbCl_2$) (Durlak et al., 1997). However an increase in moisture content decreases the levels of free chlorine, which has the subsequent effect of shifting Pb from gaseous $PbCl_2$ to PbO in particulate form. $PbCl_{2(g)}$ is completely volatilized at 430 °C (Chen and Yang, 1998; Chang et al., 1999). Above 800 C $PbCl_2$ slowly decomposes and $PbO_{(g)}$ and $PbCl_{(g)}$ are present in greater concentrations.

The presence of sodium can also affect speciation. Sodium has a greater affinity for binding with chlorine than Pb (Durlak et al., 1997). Thus, increasing the sodium content effectively shifts the dominant Pb compound from $PbCl_2$ to PbO. Decreasing the sodium content from 6560 ppm to 4500 ppm (the average value observed in municipal solid waste) was responsible for increasing Pb in the fly ash from 35% to 60% at average moisture levels (Durlak et al., 1997). High sodium concentrations attenuate the influence of moisture on Pb emissions.

Lead emissions tend to concentrate in the submicron size range (Chang et al., 1999; Olmez et al., 1988). Lead in the fine fraction was enriched by a factor of more than 10^5 at several U.S. incinerators compared with the concentration of Pb in the solid waste (Olmez et al., 1988). Lead in the coarse fraction was enriched by a factor of more than 1000.

Stationary Combustion Sources: Sewage Sludge Combustion

Sewage sludge incinerators exist at approximately 200 sites in the United States (U.S. Environmental Protection Agency, 1998). Lead can enter the sewage waste stream through car washes, galvanized material, pipe erosion, pigments, food, processed chemicals, and roofs (Krook et al., 2004). As in other combustion processes, Pb impurities vaporize during incineration and then condense.

The Pb content of dry sludge from sewage sludge incinerators varies between 80 and 26,000 ppm, with an average of 1,940 ppm (Pacyna, 1986). The emission factor for Pb from sewage sludge incineration was 140 g/ton. Sewage sludge cake taken from an industrial wastewater treatment plant in Taiwan had Pb levels of 1,500 ppm (Chang et al., 1999). Prior to combustion, Pb is either bound to organic matter in sludge or is present as a carbonate (Lockwood and Yousif, 2000).

In sewage sludge incinerators, higher temperatures are associated with higher Pb emissions (Pacyna, 1986). Additionally, sewage sludge incinerators tend to be equipped with venturi scrubbers with efficiencies of 90 to 99% (Pacyna, 1986). Other pollution control devices are less common.

Sorbents can be effective pollution controls. Kaolinite, in particular, was shown to reduce Pb emissions significantly (Lockwood and Yousif, 2000).

Emissions have been estimated as 0.14 g Pb emitted per kg of sewage sludge combusted (Pacyna, 1986). The fine fraction of particulate emissions in an experimental setup was enriched with Pb by a factor of 2.5 (Lockwood and Yousif, 2000).

Stationary Combustion Sources: Scrap Tire Combustion

Waste tires are increasingly used as a fuel, although uncontrolled burning as a result of accidents or illegal activity is common (U.S. Environmental Protection Agency, 1998). One analysis showed that uncontrolled combustion resulted in Pb emissions on the order of 0.47 mg Pb/kg tire for tires that had been cut into four to six pieces (Lemieux and Ryan, 1993). Emissions were lower for shredded tires, at 0.10 mg Pb/kg tire, probably because of greater oxygen transport between tire pieces. Another analysis detected trace amounts of Pb in the smoke from the combustion of tire bodies but did not detect Pb emissions when the tread was burned (Wagner and Caraballo, 1997).

Lead-acid Battery Manufacturing

Lead-acid batteries constituted 84% of Pb consumed in 2003, as shown in Figure 2-4 (USGS, 2003). Lead-acid batteries are manufactured from Pb alloy ingots and Pb oxide. Lead alloy ingots are produced by smelters, the emissions of which are characterized earlier in this chapter. Lead oxide is either produced on-site or is outsourced (U.S. Environmental Protection Agency, 1998). In 1975, at one facility in Pennsylvania, ambient Pb concentrations ranged from 4.1 to 5.2 $\mu g/m^3$ at several sites near the property. Lead acid battery manufacturing contributed 1.7% of Pb emissions in the U.S., or ~25 tons, in the 2002 NEI (U.S. Environmental Protection Agency, 2006a).

Lead-acid battery manufacture consists of the following processes: grid casting or stamping, paste mixing, plate stacking, group assembly, and battery assembly into the battery case (U.S. Environmental Protection Agency, 1998). Each process has its own characteristic emissions of Pb. Emissions from Pb oxide manufacture tend also to be in the form of Pb oxides. These emissions are typically controlled via a baghouse. The sites of other processes are usually equipped with baghouses or impingement wet scrubbers (U.S. Environmental Protection Agency, 1998).

Cement Manufacturing

The manufacture of Portland cement emits relatively low quantities of Pb. In the 2002 NEI, Portland cement manufacturing resulted in ~21 tons of emissions, or about 1.5% of total U.S. Pb emissions (U.S. Environmental Protection Agency, 2006a). Trace amounts of Pb are present in the raw materials of calcium, silicon, aluminum, and iron (U.S. Environmental Protection Agency, 1998). As the raw materials are thermo-treated, most of the Pb is trapped in the resulting clinker, although some is released as PM (U.S. Environmental Protection Agency, 1998). Additionally, emissions result from the combustion of the coal, natural gas, or waste tires used to fire the kiln (Pacyna, 1986; U.S. Environmental Protection Agency, 1998).

Emissions are reduced significantly through the use of pollution control devices. ESPs and baghouses are both common although baghouses tend to be more effective. Lead is present in the emitted PM in the range of 100 to 1000 ppm (Lee and Von Lehmden, 1973). Emission factors for cement production are listed in Table 2-16.

Table 2-16. Emission Factors for Processes Used in Cement Manufacture by Control Device

Process	Pollution Control Device		
	Multi-cyclones	ESP	Baghouse
Dry Process (total)	16.0	4.0	0.16
Kiln/cooler	12.0	3.0	0.12
Dryer/grinder	4.0	1.0	0.04
Wet Process (total)	12.0	3.0	0.12
Kiln/cooler	10.0	2.5	0.10
Dryer/grinder	2.0	0.5	0.02

Note: Units are g Pb/metric ton cement.

Source: Pacyna (1986).

Glass Manufacturing

The production of leaded glass emits significant quantities of Pb. Its uses primarily include Pb crystal, cathode ray tubes for televisions, and optical glasses such as binoculars,

microscopes, and telescopes (U.S. Environmental Protection Agency, 1998). Leaded glass is composed of silica sand and Pb oxide. Lead oxide concentrations in U.S.-produced leaded glass typically range between 12% and 60% but can be as high as 92% (U.S. Environmental Protection Agency, 1998).

The basic process of glass manufacturing includes blending the raw materials, melting, and forming and finishing. Lead emissions can occur during all of these processes. During blending, forming, and finishing, Pb is emitted as part of fugitive dust emissions in minor quantities (Shapilova and Alimova, 2000; U.S. Environmental Protection Agency, 1998).

The major source of Pb emissions derives from the melting process. Emissions from melting depend mostly on the amount of Pb oxide in the raw material (Shapilova and Alimova, 2000; U.S. Environmental Protection Agency, 1998). Other factors are the type and efficiency of the furnace, the waste-gas volume, the smoke-flue length, and the efficiency of pollution control devices (Shapilova and Alimova, 2000). Electric furnaces emit significantly less Pb than gas-flame furnaces. One analysis found that the rate of Pb emissions from a gas-flame regenerative furnace was more than seven times higher than the rate of emissions from a deep tank electric furnace (Shapilova and Alimova, 2000). An electrostatic precipitator is the most efficient pollution control device for glass manufacturing operations, and ESPs are between 80% and 90% effective. Wet scrubbers are relatively ineffective. Rates of Pb emissions from several types of furnaces are listed in Table 2-17.

Table 2-17. Rate of Lead Compound Emissions from Glass-Melting Furnaces

Equipment	Product	Lead Compound Emissions g/sec)
Electric tank furnace with gas-heated working zone[a]	Glass with 16% PbO	0.134
Electric tank furnace with gas-heated working zone	Glass with 16% PbO	0.002
Gas-flame potter furnace[a]	Glass with 16% PbO	0.004
Slag-lining electric furnace with gas-heated working zone	Glass with 64.5% PbO	0.004

[a] Fitted with a "cassette pulse filter" designed specifically to capture particulate emissions from small-sized, glass-melting furnaces.

Source: Shapilova and Alimova (2000).

Ammunition Production and Shooting Ranges

In 2003, 48,800 metric tons of Pb were consumed in the United States for the production of ammunition (USGS, 2003). Additionally, some Pb is used to produce Pb azide or Pb styphnate, which is a detonating agent. Small arms manufacturing plants are likely emitters of Pb although the actual quantity is unknown.

Shooting ranges, both outdoor and indoor, may have a local impact on airborne Pb concentrations. Lead is emitted both from cast Pb bullets and Pb-based primers (Gulson et al., 2002). The propellants contain <2 ppm Pb and seem to have a negligible effect on air Pb concentrations. A 97% reduction in the air Pb concentrations was observed when Cu-jacketed bullets replaced cast Pb bullets (Gulson et al., 2002). In comparing the Pb exposure of personnel, there seems to be little difference between indoor and outdoor firing ranges (Gulson et al., 2002). One study found that soil Pb concentrations at an outdoor firing range were elevated by up to 2600 times background concentrations, indicating significant atmospheric deposition (DeShields et al., 1998).

An additional source of Pb emissions may be explosive ordnance disposal (EOD) (U.S. Environmental Protection Agency, 1998). Emissions from EOD are either from the combustion or detonation of the propellant and primer material or from nonenergetic wastes such as containers and other wastes associated with the propellant (U.S. Environmental Protection Agency, 1998).

Demolition

A study of Pb dust-fall during the demolition and debris removal of urban row houses found that Pb was released in very large quantities (Farfel et al., 2003). Many of the row houses demolished at three sites in Baltimore, MD contained Pb-based paint in addition to being near sites with elevated levels of Pb in street dust (~700 ppm), sidewalk dust (~2000 ppm), and residential entryway mat dust (~750 ppm). The results of the study showed that dust fall within 10 m of the demolition sites was much higher than baseline measurements and was highly enriched with Pb (Farfel et al., 2003). The geometric mean Pb dust fall rate increased to 410 μg $Pb/m^2/hr$ during demolition and to 61 μg $Pb/m^2/hr$ during debris removal. The baseline rate was just 10 μg $Pb/m^2/hr$. The Pb concentration in dust fall was 2600 ppm during demolition,

1500 ppm during debris removal, and 950 ppm at baseline (Farfel et al., 2003). Thus, demolition and debris removal can be a major source of airborne Pb under some conditions.

Other Stationary Sources of Lead Emissions

There are additional stationary sources of Pb emissions that have not been mentioned above. Each of these sources are relatively small, but may be an important local source. Previously unmentioned Pb sources include: medical waste incineration, hazardous waste incineration, drum and barrel reclamation, crematories, pulp and paper mills, pigment production, Pb cable coating production, frit manufacturing, ceramics and glaze production, type metal production, pipe and sheet Pb production, abrasive grain processing, solder manufacturing, electroplating, resin stabilizer production, asphalt concrete production, paint application, and rubber production.

2.2.4 Mobile Sources

Automotive Sources of Lead Emissions

Lead is used to manufacture many components in on-road vehicles including the battery, bearings, paint primers, corrosion-resistant gas tanks, and some plastic and ceramic electrical components (U.S. Environmental Protection Agency, 1998). The major sources of Pb emissions—fuel combustion and vehicle wear—are considered below.

Emissions from Combustion of Unleaded Gasoline

Although its phaseout began in 1975, some Pb was still added to gasoline in the United States as an anti-knock additive at the time of the 1986 Lead AQCD. The ban on Pb additives to most U.S. motor vehicle gasoline did not fully take effect until 1995, with Pb additives continuing to be added to piston-engine aircraft fuel and gasoline for some types of race cars. Since the phaseout of Pb additives from motor vehicle fuel, U.S. airborne-Pb concentrations have fallen dramatically nationwide. This is considered one of the greatest public and environmental health successes (Nriagu, 1990). For example, airborne concentrations in the United States fell an average of 94% from 1983 to 2002 and 57% from 1993 to 2002 (U.S. Environmental Protection Agency, 2003).

Most countries have made a similar move away from leaded fuel, but a few continue the practice of adding tetraethyl Pb to automotive gasoline, particularly most countries in Africa and some in Asia. Worldwide Pb consumption for gasoline peaked in the 1970s at just under 400,000 metric tons, but by 1993, this value fell to about 70,000 metric tons (Socolow and Thomas, 1997). Leaded gasoline was the largest source of air emissions throughout the 1970s and 1980s (Socolow and Thomas, 1997). In Pakistan, a country that continues to use leaded fuel, the airborne Pb concentrations in the urban center of Karachi range between 2.0 and 19 $\mu g/m^3$ (Parekh et al., 2002). This is 2 to 3 orders of magnitude higher than typical urban concentrations in the United States.

In the absence of tetraethyl Pb additives, Pb is emitted from automobiles as a trace element in PM. Metals enter the vehicle in trace amounts, naturally occurring in gasoline and also a component of lubricating oil. The amount of PM that is emitted from the car depends on a number of variables including the ambient temperature, the cruising speed, the amount of stop-and-go activity, the type of catalyst, the fuel quality, the phase of driving, and the age, size, maintenance level, and engine type of the vehicle. EPA promulgated PM standards in 2000 for light-duty vehicles that cap PM emissions at 0.01 g/mile.

The amount of Pb that naturally occurs in gasoline is ~0.00005 g/L (Harris and Davidson, 2005). An estimated 30 to 40% of this Pb deposits in the engine and exhaust system; the balance is emitted (Huntzicker et al., 1975; Loranger and Zayed, 1994). The deposition of Pb in the engine and exhaust system varies greatly under different driving conditions, and Pb that has been retained here is re-entrained into the exhaust under certain transient operating conditions, such as heavy acceleration. Due to this complex behavior, inventory estimates of Pb from motor vehicles (e.g., those presented in Table 2-8) are derived from Pb concentrations in fuel and fuel consumption estimates and not emissions testing data. Data from engine tests of light-duty and heavy-duty vehicles are discussed below. These data are helpful in understanding the size fraction for the emitted Pb, but the values reported highlight the variability in emissions and large uncertainties in measurements made. Lead is also present in lubricating oil, and concentrations can vary widely. However, since EPA does not regulate Pb in lubricating oil, no systematic data have been collected or are now available on Pb levels in lubricating oil.

Particulate matter emissions have been shown to be higher in older vehicles than in newer vehicles (Gillies et al., 2001; Cadle et al., 1999). Gillies et al. (2001) compared emission factors

from several studies, and found that emission factors from car models between the years 1964 and 1983 had emission factors for PM that were about an order of magnitude higher than models from the 1990s. This was true even of catalyst-equipped vehicles. Similarly, Cadle et al. (1999), using unleaded gasoline, tested 195 cars with model years between 1971 and 1996. Their results, which are listed in Table 2-18, show an increase in Pb emission rates with automobile age.

Table 2-18. Lead Emission Factors During Summer Versus Winter for Automobiles with Model Years Between 1971 and 1996

Vehicle Category	Emission Factors in mg/mile	
	Summer	Winter
1991-1996	0.003	0.019
1986-1990	0.027	0.019
1981-1985	0.006	0.103
1971-1980	0.043	0.222
Smokers	0.035	0.282
Diesel	0.15	0.142

Note: "Diesel" denotes diesel automobiles, "Smokers" denotes automobiles with visible emissions.

Source: Cadle et al. (1999).

Vehicles that have visible tailpipe emissions are known as "smokers." The emissions of almost all pollutants are elevated from smoking vehicles compared to their non-smoking counterparts. Emission rates of Pb from smokers are an order of magnitude higher than typical cars manufactured in the 1990s, as shown in Table 2-18. Interestingly, another study found that smokers and other high-emitting vehicles emitted more Pb after undergoing repair than before (Cadle et al., 1997). The emission rate of Pb before repair had an average value of 0.03 mg/mi with a standard deviation of 0.05 mg/mi. After repair, the emission rate for Pb increased to 0.16 mg/mi with a standard deviation of 0.5 mg/mi. The authors explain this surprising result by suggesting that either changes in combustion conditions caused elemental deposits from the engine and exhaust system to be released, or PM deposited during repair and testing was not removed before emissions testing (Cadle et al., 1997).

Table 2-18 also shows the effect of the ambient temperature on emission rates of Pb. Emissions tend to be higher during cold months than during warm months (Cadle et al., 1999).

The rate of emissions is largely dependent on the phase of driving. The Federal Test Procedure analyzes three phases: cold start, hot stabilized, and hot start, the results of which are shown in Table 2-19. Driving cycles that are not included are the highway fuel economy test, and a high speed, high load cycle known as US06 (Cadle et al., 1999). Emissions were significantly higher during cold start than during the hot stabilized and hot start phases.

Table 2-19. Lead Emission Factors for Different Driving Phases for Automobiles with Model Years Between 1971 and 1996

Vehicle Category	Summer Emission Factors in mg/mile		
	Cold Start	Hot Stabilized	Hot Start
1991-1996	0.005	0.002	0.002
1986-1990	0.041	0.020	0.031
1981-1985	0.016	0.002	0.006
1971-1980	0.112	0.015	0.044
Smokers	0.116	0.010	0.031
Diesel	0.190	0.048	0.313

Source: Cadle et al. (1999).

Despite the large variability in Pb emissions, several studies describe average on-road emission factors for a typical fleet. Sternbeck et al. (2002) measured metal concentrations in two tunnels in Gothenburg, Sweden. The emission factors subsequently derived were 0.036 ± 0.0077 mg/km per vehicle and 0.035 ± 0.014 mg/km per vehicle for the two tunnels, respectively. Another tunnel study was performed on a fleet comprised of 97.4% light-duty vehicles and 2.6% heavy-duty vehicles in the Sepulveda Tunnel in California (Gillies et al., 2001). The emission factors for Pb were 0.08 mg/km per vehicle and 0.03 mg/km per vehicle in the PM_{10} and $PM_{2.5}$ fractions respectively. Lough et al. (2005) analyzed emissions from on-road vehicles in two tunnels in Milwaukee, Wisconsin. Trucks constituted between 1.5% and 9.4% of the vehicles, with the balance comprised of passenger cars. Lead emission rates were on the order of 0.01 mg/km per vehicle and 0.1 mg/km per vehicle in the summer and winter

respectively. Cadle et al. (1999) analyzed 195 in-use, light-duty vehicles using two dynamometers. Their results are shown in Tables 2-18 and 2-19. A test on noncatalyst-equipped, light-duty vehicles found that Pb constituted about 0.03% of the fine particle mass emitted from these vehicles (Kleeman et al., 2000).

Vehicle-derived Pb seems to have a bimodal size distribution. The submicron mode is likely the product of combustion or high temperatures, and therefore probably came from the tailpipe (Lough et al., 2005; Harrison et al., 2003; Abu-Allaban et al., 2003). The coarse mode, with an approximate size range of 1.0 to 18 μm in diameter, is likely a product of physical processes such as road dust resuspension and tire or brake wear (Lough et al., 2005; Abu-Allaban et al., 2003). More than 80% of the airborne Pb particles near a roadway were $<PM_{2.5}$ (Harrison et al., 2003).

Emissions from Combustion of Diesel Fuel

In on-road studies of a typical fleet, as in tunnel studies, the relative contributions of diesel fuel and gasoline are difficult to separate. Emissions of PM from diesel vehicles are highly dependent on the mode of operation (Shah et al., 2004). Emission rates are much higher in simulated congested traffic situations than at cruise or highway speed conditions (Shah et al., 2004). Extensive profiles of diesel emissions were developed by Lowenthal et al. (1994). Their results for Pb are summarized in Table 2-20.

Particulate matter from diesel vehicles tends to be smaller than $PM_{2.5}$ (Gillies et al., 2001; Kleeman et al., 2000). The peak of the particle mass distribution appears to be around 0.1 μm (Kleeman et al., 2000). Although no data were available specifically for Pb, such small particle sizes would be consistent with expectations for particle formation from high-temperature processes.

Emissions from Vehicle Wear

Vehicle wear and loss of Pb wheel weights are also considered to be sources of roadside Pb contamination. Brake wear, in particular, may emit significant quantities of Pb in PM. Harrison et al. (2003) note that Pb is poorly correlated with emissions of NO_x, which is emitted from tailpipes. These authors therefore suggest that brake wear likely contributes the additional quantities of Pb observed in ambient air. Sternbeck et al. (2002) compared emission factors

Table 2-20. Lead Concentration in Particulate Matter Emissions and Lead Emissions Factors for Buses and Trucks Fueled with Diesel No. 2 and Jet A Fuel

Fuel and Vehicle Type	Concentration of Pb in PM (%)	Uncertainty (%)	Emission Factor (mg/km)	Uncertainty (mg/km)
Truck, Diesel No. 2	0.0007	0.0028	0.0053	0.0187
Truck and Bus, Diesel No. 2	0.0006	0.0025	0.0045	0.0188
Truck and Bus, Jet A	0.0010	0.0055	0.0050	0.0214
Bus, Jet A and Diesel No. 2 with particulate trap	0.0009	0.0052	0.0016	0.0100
Bus, Jet A with particulate trap	0.0028	0.0132	0.0018	0.0085
Phoenix PM_{10} study	0.0147	0.0294	n.a.	n.a.

The results of Chow et al. (1991) on heavy-duty particulate emissions in Phoenix are listed in the last row for comparison.

Source: Lowenthal et al. (1994).

derived in other studies. Estimates of Pb emissions from brake pads in Sweden were just under 200 µg/km per vehicle (Sternbeck et al., 2002). This is an order of magnitude higher than the tailpipe emissions measured by Cadle et al. (1999).

Up to 35% of brake pad mass loss is emitted as airborne PM (Garg et al., 2000). One study that analyzed particulate emissions from seven different brake pad formulations found that only one type of brake pad described as "potassium titanate, aramid, and copper fiber" emitted PM with a measurable Pb fraction (Garg et al., 2000).

A joint study in Reno, NV and Durham/Research Triangle Park, NC found that the dominant contributors to roadside PM were resuspended road dust and tailpipe emissions (Abu-Allaban et al., 2003). However, brake wear was a significant source of PM in places where strong braking occurred, such as at freeway exits (Abu-Allaban et al., 2003).

Particulate matter emissions from brake pads were primarily in the fine fraction. Eighty-six percent and 63% of airborne PM was smaller than 10 and 2.5 µm, respectively (Garg et al., 2000). It is expected that Pb particles from mechanical processes such as brake wear would be in the coarse fraction. However, smaller particles may be observed if Pb is vaporized from hot brake surfaces (Harrison et al., 2003; Lough et al., 2005).

Lead weights used to balance vehicle wheels may be an additional source of elevated roadside Pb concentrations. Deposition of Pb from wheel weights at one intersection in Albuquerque, NM was estimated to be between 50 and 70 kg/km per year (Root, 2000). Wheel weights are 95% Pb, 5% antimony, and typically weigh between 7 and 113 grams. These wheel weights can become dislodged during quick stops. Although deposited pieces of wheel weights are quite large, Pb is very malleable and can be worn away into respirable particles by being run over by vehicles (Root, 2000).

Emissions from Racing Vehicles

Vehicles used in racing (including cars, trucks, and boats) are not regulated by the EPA according to the Clean Air Act and can therefore use alkyl-lead additives to boost octane. Data on Pb levels in racing fuel and rates of Pb emissions are scarce. The U.S. Department of Energy stopped tracking information on the production of leaded gasoline for non-aviation use in 1990 (U.S. Environmental Protection Agency, 2002). However, the National Motor Sports Council reports that ~100,000 gallons of leaded gasoline were used by National Association for Stock Car Automobile Racing (NASCAR) vehicles in 1998 (U.S. Environmental Protection Agency, 2002).

As was the case with on-road emissions during the time of universal leaded gasoline use, the combustion of racing fuel likely elevates airborne Pb concentrations in the nearby area. This may pose some health risk for some subpopulations, such as residents living in the vicinity of racetracks, fuel attendants, racing crew and staff, and spectators. However, EPA has formed a voluntary partnership with NASCAR with the goal of permanently removing alkyl-Pb from racing fuels used in the Busch, Winston Cup, and Craftsman Truck Series (U.S. Environmental Protection Agency, 2002). In January of 2006, NASCAR agreed to switch to unleaded fuel in its racecars and trucks beginning in 2008.

In addition to racing vehicles and piston engine aircraft, legally permitted uses of leaded fuel include construction machinery, agricultural equipment, logging equipment, industrial and light commercial equipment, airport service equipment, lawn and garden equipment, and recreation equipment, including boats, ATVs, jet skis, snowmobiles, etc. (U.S. Environmental Protection Agency, 2000). Given the relative unavailability of leaded fuel, it is unlikely that it is commonly used for any of these purposes other than racing vehicles.

Emissions from the combustion of leaded fuel are generally in the form of submicron particles of inorganic Pb halides.

Aircraft

Piston-engine aircraft use leaded fuel. Aviation fuel, or "avgas," contains 0.1 to 1.0 g of tetraethyl Pb additives per liter. About 32.7% of general aviation aircraft use avgas, and the remainder use jet fuel, which does not contain Pb additives (U.S. FAA, 1996). The overall fraction of aviation fuel containing Pb additives is unknown.

In the South Coast Air Basin of California, emissions of Pb from general aviation aircraft have been estimated at 634 ± 110 kg/year (Harris and Davidson, 2005). This corresponds to 0.54 grams of Pb released per flight. Approximately 267 kg of the total was emitted below the mixing height in 2001, which could be a local source of Pb exposure.

Commercial jet aircraft do not use leaded fuel. However, they are also likely sources of some Pb emissions. In-flight sampling of contrails from a DC-8 and a 757 showed that metals constituted more than 11% and 5.2% of PM, respectively (Twohy and Gandrud, 1998). This is a lower limit for the fraction of metals in emissions since almost half of the particles in contrails are from the ambient air (Twohy and Gandrud, 1998). No known estimates have been made of the quantity of Pb in commercial aircraft emissions. However, the dominant metals seem to be Fe, Cr, and Ni (Kärcher, 1999). These are the primary components of stainless steel and indicate that aircraft engine erosion may be a significant source of metal emissions (Kärcher, 1999).

Metal particles in contrails have two modes. One is submicron with an average diameter of about 0.36 μm (Kärcher, 1999; Twohy and Gandrud, 1998), whereas the other larger mode is ~1 μm in diameter and has a morphology that suggests mechanical generation (Kärcher, 1999).

Lawn-Care Equipment

A life cycle assessment used to compare gasoline-, electricity-, and battery-powered lawn mowers found that electricity-powered mowers had the fewest overall emissions over their lifetime (Sivaraman and Lindner, 2004). Battery-powered mowers are fitted with a lead-acid battery. The total amount of Pb released to the environment a typical Pb-acid battery over its lifetime averages ~0.052 kg Pb, which includes consideration of raw material extraction and refining, energy production, Pb mining and refining, battery manufacture, and battery recycling

(Sivaraman and Lindner, 2004). Electricity-powered lawn mowers presumably emit less PM and Pb than gasoline-powered mowers. This is a reasonable assumption, since utility generation plants tend to be fitted with pollution control devices and internal combustion engines of gasoline-powered mowers do not.

Other Sources of Lead Emissions

Lead emissions are associated with the combustion of any fossil fuel. Thus, any of the following may be additional types of mobile Pb emission sources not separately addressed above: construction equipment, off-road recreational vehicles, generators, marine vessels, railroad locomotives, agricultural equipment, logging equipment. However, detailed data on Pb emissions from such sources are not readily available.

Additionally, the resuspension of Pb-contaminated soil and dust is a major source of airborne Pb. Since fugitive dust emissions are not considered a primary source of airborne Pb a discussion of resuspended soil particles is omitted here and covered in Section 2.3.3 as a mode of transport for Pb particles through the environment.

2.3 TRANSPORT WITHIN THE ENVIRONMENT

2.3.1 Atmospheric Transport of Lead Particles

Atmospheric Dispersion

The atmosphere is the major environmental transport pathway for anthropogenic Pb (Reuer and Weiss, 2002). Airborne Pb tends to be mainly in the form of submicron aerosols (Davidson and Rabinowitz, 1992; Davidson and Osborn, 1986; Harrison, 1986; Lin et al., 1993). The mass median diameter averaged for several studies is 0.55 µm (Milford and Davidson, 1985). A study performed in 1991, after leaded gasoline was no longer the predominant source of Pb in the atmosphere, showed a bimodal distribution for Pb particles, with the larger peak in the fine fraction (Lin et al., 1993). The mass median diameter for Pb samples was 0.38 ± 0.06 µm in the fine fraction and 8.3 ± 0.6 µm in the coarse fraction. Since small particles are much slower to deposit than larger particles, Pb can be transported great distances in the atmosphere. Detectable quantities of Pb have been found even in the most remote places on earth. Because much of the airborne Pb is generally associated with fine particles, atmospheric

dispersion models used for gaseous pollutants can be applied to estimate atmospheric flows of Pb under certain conditions. Use of such dispersion models is more accurate for submicron Pb emitted from stacks than it is for larger particles resulting from fugitive emissions, such as resuspended soil particles.

Airborne concentrations of species emitted from a point source are frequently described by a Gaussian distribution. This simple description holds true only when turbulence is stationary and homogeneous. However, the Gaussian model can be modified to account for more complex atmospheric conditions. For a thorough discussion of assorted Gaussian plume models and parameters, see Seinfeld and Pandis (1998). Gaussian models are, in general, reasonably accurate for small-scale work, i.e., within ~100 km of the source.

The rate and direction of dispersion are dependent both on pollutant characteristics and meteorological conditions. Important meteorological factors include windspeed, surface roughness, inversion frequency, inversion duration, and temperature.

A Gaussian dispersion model (EMITEA-AIR) was applied to theoretical primary and secondary Pb smelters in Europe (Baldasano et al., 1997). This model accounts for plume rise, as well as interactions between the plume and terrain. Two sites were modeled. Conditions in Copenhagen, Denmark included flat terrain, dominant strong winds, neutral or stable turbulence, and an annual mean temperature of 10°C. Conditions in Catalunya, Spain had a complex terrain, weak winds, unstable turbulence, and an annual mean temperature of 15°C.

The results of these modeling efforts showed that airborne Pb concentrations were both lower and more symmetric surrounding the Copenhagen site than surrounding the Catalunya site (Baldasano et al., 1997). Concentrations at the Copenhagen site had a maximum value of 0.004 $\mu g/m^3$. Concentrations at the Catalunya site ranged between 0.065 and 0.3 $\mu g/m^3$. The prevalence of calm winds and the complex terrain were the most important factors contributing to high Pb concentrations surrounding the Catalunya smelter.

Modeling efforts for an abandoned battery recycling facility using the EPA Industrial Source Complex Short Term (ISCST) model, based on Gaussian equations, showed good agreement with measured airborne Pb concentrations (Small et al., 1995). Model predictions at three sites at distances between 240 and 310 m from the stack were between 3.8 and 4.4 $\mu g/m^3$, whereas measured Pb concentrations taken when the plant was in full operation had averages between 4.1 and 5.2 $\mu g/m^3$.

For long-range transport modeling, Lagrangian trajectory or Eulerian grid models are commonly employed. These models determine how a parcel of air moves relative to the moving fluid and a fixed coordinate system, respectively. Two Lagrangian experiments were performed in the Azores in the northern Atlantic (Véron and Church, 1997). Retrospective air mass trajectories based on the hybrid single-particle Lagrangian integrated trajectory (HY-SPLIT) model found that air masses enriched with Pb had been over continental regions ten days prior to testing. This is consistent with current understanding that most Pb emissions are from sources on continents, not from oceanic sources. Airborne Pb at this remote location was transported from several different countries (Véron and Church, 1997).

Similarly, backward air mass trajectories estimated for Greenland showed that the highest air concentrations of metals were in air parcels that had been over continental regions five days earlier (Davidson et al., 1993). The model used in this study employed a constant acceleration formulation of the trajectory equations and encompassed air parcel movements affected by terrain and meteorology. The air masses with the highest metal concentrations were traced back to polluted regions, including the Arctic Basin, eastern North America, and Western Europe (Davidson et al., 1993).

A numerical model that combined weather system modeling with three-dimensional Lagrangian transport and diffusion modeling was used to determine the foreign contributions of Pb to airborne concentrations in Israel (Erel et al., 2002). These predictions, in conjunction with isotopic measurements, indicated that Israel received significant amounts of Pb from Egypt, North Africa, the United Arab Emirates, Jordan, Turkey, and Eastern Europe (Erel et al., 2002).

Historical Records of Atmospheric Lead Transport and Deposition

An important field of research involves analyzing natural records of Pb deposited from the atmosphere. Lead concentrations are measured in media such as soil, sediments, ocean water, peat bogs, plants, snowpacks, or ice cores. Based on concentrations, ratios to other pollutants, or isotopic compositions, an airborne concentration is back calculated and, in some cases, the major emitters can be identified. Sediments can provide records dating back several million years, peat bogs can reach back to the late glacial period (~15000 years ago), corals and trees can record up to several hundred years, and lichens and mosses can provide recent deposition data (Weiss

et al., 1999). Additionally, some applications can yield data showing variation with seasons or climate. These methods have been used to monitor both short and long-range transport.

Many studies have shown a pattern of sediment Pb concentrations increasing to reach peak concentrations in layers representing deposition during the 1970's followed by marked declines in more recent years. For example, Figure 2-4 presents data on Pb concentrations in sediment samples from 12 lakes in the Great Lakes area (Yohn et al., 2004). Other such studies have been conducted in the Okefenokee Swamp in Georgia (Jackson et al., 2004), Lake Clair in Quebec (Ndzangou et al., 2005), in two ponds near a Superfund site in Massachusetts (Norton et al., 2004), lake sediments near Sudbury, Ontario (Belzile et al., 2004), several Canadian shield lakes (Gallon et al., 2004; Gallon et al., 2006), and several lakes in Scotland (Eades et al., 2002). A similar pattern has been seen in peat cores from three Southern Ontario bogs (Givelet et al., 2003) and two peat bogs in Spain (Cortizas et al., 2002), and peat deposits in Switzerland (Shotyk, 2002). Nieminen et al. (2002) show a pattern of declining Pb concentrations with greater height in peat cores at a "background" site, but markedly higher Pb concentrations in peat cores collected near a Cu/Ni smelter in Southwest Finland. For a comprehensive look at natural historical records, the reader is referred to review articles by Weiss et al. (1999), Boutron et al. (1994), and Garty (2001).

2.3.2 Deposition of Airborne Particles

Deposition (both dry and wet) is the major removal mechanism for atmospheric pollutants and atmospheric deposition can be a major source of Pb into lakes (Balistrieri et al., 1995). Here the main focus is on deposition data published specifically for Pb aerosols, although the literature on particle deposition is extensive.

Dry Deposition

Dry deposition is the process by which pollutants are removed from the atmosphere in the absence of precipitation. The downward flux, -F, is characterized by:

$$-F = V_d C \qquad (2\text{-}5)$$

where C is the airborne concentration in $\mu g/m^3$ and V_d is the deposition velocity in m/second. The deposition velocity is an empirical quantity defined by Equation 2-5 as the ratio of F to C

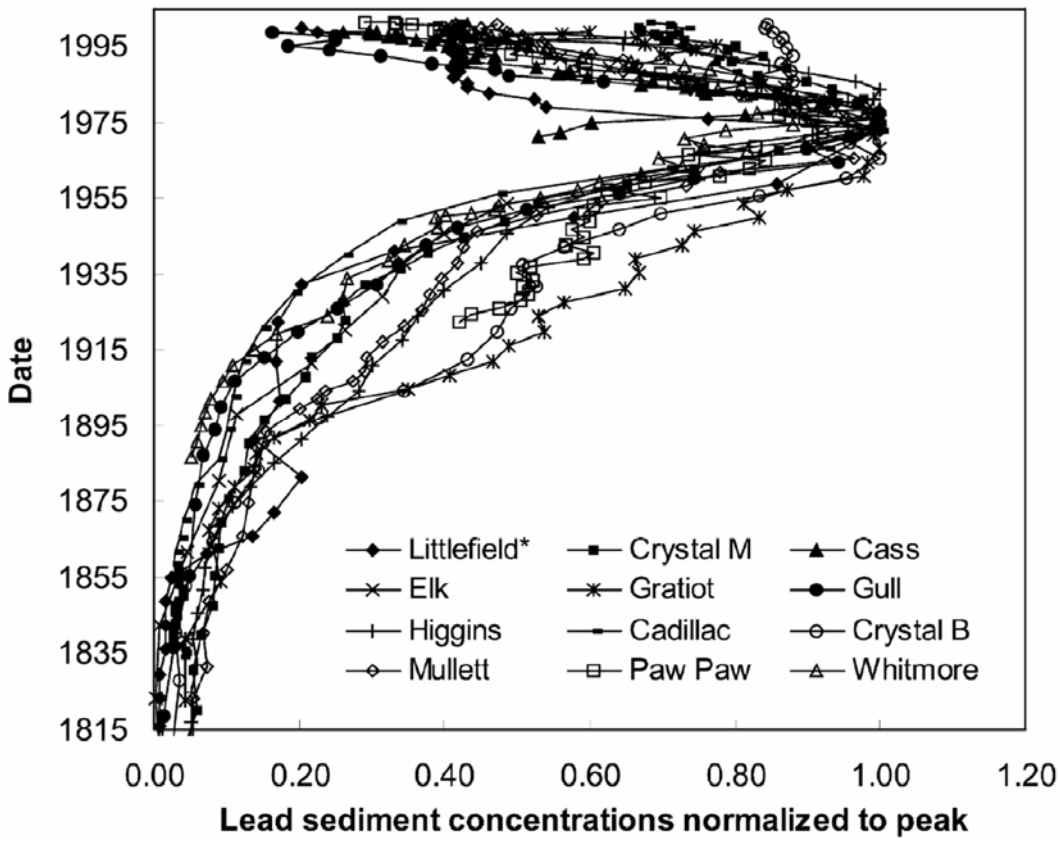

Figure 2-4. Lead concentrations in sediment samples in 12 Michigan lakes. The concentrations are normalized by the peak Pb concentration in each lake; peak Pb concentrations ranged from approximately 50 to 300 mg/kg.

Source: Yohn et al. (2004).

with units of m/s. It should be noted that both the airborne concentration and the deposition velocity are dependent on vertical height.

The physical factors governing dry deposition are often described in a manner analogous to electronic resistances (Davidson and Wu, 1990). The parameters of aerodynamic resistance, boundary layer resistance, and surface resistance run in parallel with sedimentation resistance or gravity. The relative importance of each of these resistances varies with particle size and meteorological conditions (Wu et al., 1992a).

The size of depositing particles is arguably the most important factor affecting deposition rates. For very small particles, Brownian motion is the dominant mechanism that transports particles through the viscous sublayer that borders surfaces (Nicholson, 1988a). For large

particles, sedimentation is the most important process governing particle deposition. For intermediate particles, impaction and interception largely determine deposition rates. The deposition velocity has the most uncertainty for these intermediate sized particles (Nicholson, 1988a). Although most of the airborne Pb mass was associated with submicron particles, only about 0.5% of the Pb particle mass undergoing dry deposition in Chicago was <2.5 µm in diameter (Lin et al., 1993). Additionally, more than 90% of Pb particle mass that undergoes dry deposition is in an insoluble chemical form (Gatz and Chu, 1986).

Deposition velocities for Pb are in the range of 0.05 to 1.3 cm/s. Table 2-21 is a compilation of data from the literature. Figure 2-5 shows the variation of deposition velocity for Pb as a function of particle size. Dry deposition flux values have been reported for several studies. An estimated flux of 370 to 1000 µg/m^2-year (~0.37-1.0 mg/m^2-year) was based on data collected from two sites on Chesapeake Bay during 1990-1991 (Wu et al., 1994). The mean air Pb concentration associated with $PM_{2.5}$ over an estuary in the New York-New Jersey Harbor Bight area was 4.9 ng/m^3, and dry deposition flux associated with $PM_{2.5}$ was estimated to be 0.15-0.76 mg/m^2-year (Gao et al., 2002). Dry deposition flux at several sites near the Lake Michigan ranged from 0.023 to 0.038 mg/m^2-day (8.4-14 mg/m^2-year) using data collected in 1993-1995 (Yi et al., 2001). In seven urban sites across the metropolitan Detroit area, estimates of dry deposition flux rates decreased by about an order of magnitude from 1982 to 1991, from nearly 10 to below 1 g/km^2-day (nearly 4 to below 0.4 mg/m^2-year) (Pirrone et al., 1995). Dry deposition flux of 7.0 µg/m^2-day (2.6 mg/m^2-year) was estimated over the Ligurian Sea (Migon et al., 1997). Total deposition flux of Pb (in fine and coarse fraction particles) into Lake Erie was 43.3 ng/m^2-hour in April 1992 (Keeler and Pirrone, 1996). In an industrial area of northern France, dry deposition of Pb was estimated to be 40 to 80 µg/m^2-hour (Franssens et al., 2004). Based on Pb in both fine and coarse particles, dry deposition was estimated to be 920 µg/m^2-year (0.920 mg/m^2-year) in Lake Superior, 950 µg/m^2-year (0.950 mg/m^2-year) in Lake Michigan, and 780 µg/m^2-year (0.780 mg/m^2-year) in Lake Erie (Sweet et al., 1998).

Wet Deposition

Wet deposition is the process by which airborne pollutants are scavenged by precipitation and removed from the atmosphere. The flux of a depositing species can be defined through the following equation:

Table 2-21. Dry Deposition Velocities for Lead Particles

Vd (cm/s)	MMAD (µm)	Surface	Other	Reference	
0.26	all	water		Davidson and Rabinowitz (1992)	
0.56	all	orchard grass		Davidson and Rabinowitz (1992)	
0.06 ± 0.02	all		model of Rojas et al. (1993)	Rojas et al. (1993)	
0.06 ± 0.02	all		model of Slinn and Slinn (1980)	Rojas et al. (1993)	
0.09 ± 0.03	all		model of Williams (1982)	Rojas et al. (1993)	
0.26	all	all	mass balance model	Friedlander et al. (1986)	
0.14 ± 0.13	10% >4	teflon plates		Davidson and Wu (1990)	
0.15 ± 0.07	0.87	teflon plates		Davidson et al. (1985)	
0.41	0.68	water		Davidson and Wu (1990)	taken from Dedeurwaerder et al. (1983)
0.43	0.75	water		Davidson and Wu (1990)	taken from Dedeurwaerder et al. (1983)
0.19	0.70	land		Davidson and Wu (1990)	taken from Dedeurwaerder et al. (1983)
0.33 ± 0.03		alfalfa + oil	stable conditions	El-Shobokshy (1985)	
0.31 ± 0.02		alfalfa + oil	unstable conditions	El-Shobokshy (1985)	
0.37 ± 0.04		grass + oil	stable conditions	El-Shobokshy (1985)	
0.31 ± 0.02		grass + oil	unstable conditions	El-Shobokshy (1985)	
0.28 ± 0.05		soil	stable conditions	El-Shobokshy (1985)	
0.34 ± 0.05		soil	unstable conditions	El-Shobokshy (1985)	
0.9 ± 0.3	0.79	beech canopy	throughfall	Davidson and Wu (1990)	taken from Höfken et al. (1983)
1.3 ± 0.5	0.79	spruce canopy	throughfall	Davidson and Wu (1990)	taken from Höfken et al. (1983)
0.05	0.5	polyethylene petri dish		Davidson and Wu (1990)	taken from Lindberg and Harriss (1981)
0.005	0.5	oak	foliar extraction	Davidson and Wu (1990)	taken from Lindberg and Harriss (1981)
0.06 ±0.01	0.5	polyethylene petri dish		Davidson and Wu (1990)	taken from Lindberg and Harriss (1981)
0.46		filter paper		Davidson and Wu (1990)	taken from Pattenden et al. (1982)
0.06	0.3	bucket		Davidson and Wu (1990)	taken from Rohbock (1982)
0.13	82%<1	water	aerometric mass balance	Davidson and Wu (1990)	taken from Sievering et al. (1979)
0.27-0.74		water		Wu et al. (1994)	

Source: Davidson and Rabinowitz (1992), Rojas et al. (1993), Friedlander et al. (1986), Davidson and Wu (1990), Davidson et al. (1985), and El-Shobokshy (1985), Wu et al. (1994).

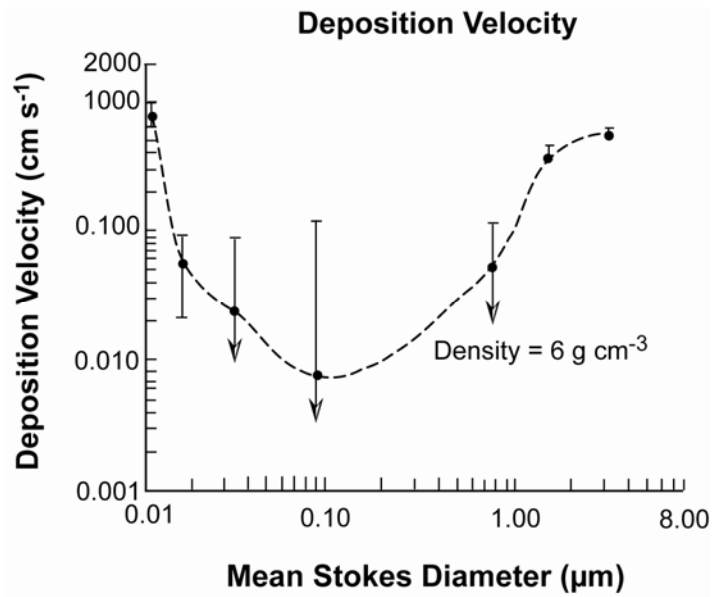

Figure 2-5. The deposition velocity plotted against the geometric mean Stokes diameter for particles with a density of 6 g/cm^{-3} (i.e., lead). Error bars are shown and the arrow indicates a negative value for the lower limit of uncertainty.

Source: Reprinted from Main and Friedlander (1990).

$$F = V_p C_p \tag{2-6}$$

where V_p is the rate of precipitation in cm/s and C_p is the concentration of the chemical species in the precipitation in µg/L (Miller and Friedland, 1994).

The size of particles can influence wet deposition rates. Large particles are scavenged more efficiently. Lead, which is found primarily in the submicron size range, does not undergo wet deposition as easily as many of the crustal elements (Davidson and Rabinowitz, 1992).

Conko et al. (2004) note a seasonal trend in wet deposition rates for Pb in Reston, VA. The annual deposition rate was 440 µg/m^2-year. The highest concentrations were observed in the summer months, which the authors attribute to increased emissions from electric power plants. Wet depositional flux of Pb in 1991-1996 was found to be 51 ng/cm^2-year (510 µg/m^2-year) at a site along the Chesapeake Bay, 39 ng/cm^2-year (390 µg/m^2-year) at a site at the mouth of Delaware Bay (Kim et al., 2000), 3.1 µmol/m^2-year (642 µg/m^2-year) in two

streams in western Maryland in 1997-1998 (Lawson and Mason, 2001), and ranged from ~300 to 600 μg/m^2-year at four sites in North-central Maryland (Scudlark et al., 2005). Concentrations of Pb in rainwater in Massif Central in France ranged from 1.30 to 465 μg/L in 1994-1995 with a mean of 50.2 μg/L and an estimated annual flux of Pb from rainwater of 15.4 mg/m^2-year (Roy and Negrel, 2001). In Lake Superior, Lake Michigan, and Lake Erie, respectively, Sweet et al. (1998) estimated wet deposition rates of 550 μg/m^2-year, 640 μg/m^2-year, and 1000 μg/m^2-year.

Precipitation activity has been linked to variability in wet deposition rates. Intense rain showers had lower Pb concentrations than slow, even rainfalls (Chow, 1978). Thunderstorms typically did not have detectable quantities of Pb but occasionally produced very high levels. The Pb concentration in rainfall does not appear to be correlated to the amount of time between rainfalls, but meteorological conditions such as a thermal inversion preceding a rainfall may affect the Pb content (Chow, 1978).

Lead in rainwater includes both dissolved and particulate material. Approximately 83% of Pb in wet deposition samples was in a soluble form, compared to less than 10% in dry deposition samples (Gatz and Chu, 1986).

Typical Pb concentrations in precipitation are listed in Table 2-22. The table shows a pronounced downward trend with time presumably due to the phaseout of leaded fuel. A trend of reduced Pb concentrations in precipitation was shown in data from Lewes, DE, where average concentrations declined ~3 μg/L in 1982 to <1.0 μg/L in 1989 (Scudlark et al., 1994).

Bulk Deposition

Bulk deposition is the rate of dry and wet deposition combined. It is typically sampled in open buckets or other open containers. This is often used to estimate the overall rate of atmospheric input to soil, surface water, or other terrestrial media. However, it is understood that dry deposition onto surrogate surfaces may differ greatly from dry deposition onto natural surfaces. Gélinas and Schmit (1998) reported a bulk (wet and dry) deposition rate of 2.61 mg/m^2-year in an agricultural area near Montreal in 1993-1995. In this study, the maximum fluxes were observed in the spring and fall for PM (due to agricultural tilling practices) and lowest rates were seen in winter and summer. The authors suspect that this is due to decreased traffic in the summer months. The average deposition rates (wet + dry) estimated for Pb in the Massachusetts Bay in 1992-1993 were 2700 μg/m^2-year (Golomb et al., 1997). Total (dry and

Table 2-22. Lead Concentrations in Rainwater in the United States

Dates of Testing	Precipitation concentration (µg/L)	Cloudwater concentration (µg/L)	Location	Source
1966-1967	32.7	98.1	Northeastern US	Lazrus et al. (1970)[a]
1971-1972	31.2	93.6	Northeastern US	Schlesinger and Reiners (1974)[a]
1975-1976	25.2	75.6	Northeastern US	Smith and Siccama (1981)[a]
1977-1978	15.6	46.8	Northeastern US	Smith and Siccama (1981)[a]
1982	17.0	51	Northeastern US	Scherbatskoy and Bliss (1984)[a]
pre-1982	44 5.4-147	n.a.	Urban areas	Galloway et al. (1982)[b]
pre-1982	12 0.59-64	n.a.	Rural areas	Galloway et al. (1982)[b]
pre-1982	0.09 0.02-0.41	n.a.	Remote areas	Galloway et al. (1982)[b]
1982	~3	n.a.	Lewes, DE	Scudlark et al. (1994)
1988-1989	1.9	5.4	Northeastern US	Miller and Friedland (1991)[a]
1989	~1	n.a.	Lewes, DE	Scudlark et al. (1994)
1993-1994	0.7 ± 0.4	n.a.	Lake Superior	Sweet et al. (1998)
1993-1994	0.9 ± 0.6	n.a.	Lake Michigan	Sweet et al. (1998)
1993-1994	1.1 ± 0.8	n.a.	Lake Erie	Sweet et al. (1998)
1998	0.47 ± 0.55	n.a.	Reston, VA	Conko et al. (2004)
1998	0.76	0.58	Mt. Mansfield, VT	Lawson et al. (2003)
1998	0.54	5.45	Mt. Mansfield, VT	Malcolm et al. (2003)

Source: [a] Cited in Miller and Friedland (1994), [b] cited in Davidson and Rabinowitz (1992), and Conko et al. (2004).

wet) deposition flux to the Rouge River watershed was estimated to be 3167 g/km-year (3167 µg/m^2-year) from 1982 to 1992; the deposition flux rate declined sharply across this time period (Pirrone and Keeler, 1996). In an area influenced by many stationary sources as well as motor vehicle emissions in Paris, France, deposition flux was 39 mg/m^2-year in 1994-1995, 9.5 mg/m^2-year in 1999-2000, and slightly lower in 2001-2002 (Azimi et al., 2005).

The ratio of dry to wet deposition has been estimated to be 1.5, 0.4, and 0.25 in marine, rural, and urban areas respectively (Galloway et al., 1982). The estimated ratio of dry deposition to wet deposition ranged between 0.1 and 0.5 in arctic regions (Davidson and Rabinowitz, 1992).

Lindberg et al. (1982) reported a ratio of 0.8 for wet to dry deposition in the Tennessee Valley. In a literature survey, Hicks (1986) found that this ratio varied between 0.4 and 1.8.

Deposition can be influenced by the extent of vegetation cover. Scudlark et al. (2005) reported that dry deposition comprises $\leq$50% of total atmospheric Pb deposition in the Chesapeake Bay area, with the greatest contribution from wet deposition in winter during reduced leaf cover.

2.3.3 Resuspension of Lead-Containing Soil and Dust Particles

The resuspension of soil-bound Pb particles and contaminated road dust can be a significant source of airborne Pb in areas near major sources of Pb emissions. Resuspension by wind and vehicular traffic is emphasized here, although resuspension through other mechanical processes (such as pedestrian traffic, agricultural operations, construction, and even raindrop impaction) is possible. In general, mechanical stresses are more effective at resuspending particles than wind (Sehmel, 1980; Nicholson, 1988b).

Rapid calculations of ambient, respirable concentrations of Pb from resuspension can be performed through the use of fugitive dust emission factors. The emission rate of a pollutant as PM_{10} can be estimated through the following equation (Cowherd et al., 1985):

$$R_{10} = \alpha E_{10} A \tag{2-7}$$

where R_{10} is the emission rate of a contaminant as PM_{10} (units of mass/time), α is the fraction of contaminant in the PM_{10} size range (mass/mass), E_{10} is the PM_{10} emission factor (mass/source extent), and A is the source extent (in source dependent units, which are typically area but can be volume).

Emission factors for fugitive dust depend on whether the predominant force of resuspension is traffic or other mechanical disturbance, or wind. Emission factors are not recommended for detailed calculations but can provide order of magnitude assessments with minimal effort. Condition specific equations for fugitive dust emissions are provided by Cowherd et al. (1985) and AP-42 (U.S. Environmental Protection Agency, 2005).

Understanding the physics of resuspension from natural winds requires analyzing the wind stresses on individual particles, including frictional drag, form drag, gravitation, and the

Bernoulli effect (Sehmel, 1980). Although this analysis can be accurate on a small scale, predicting resuspension on a large scale generally focuses on empirical data for continual soil movement due to three processes: saltation, surface creep, and suspension (Sehmel, 1980; Nicholson, 1988b). Saltation is the process by which particles in the 100 to 500 μm size range bounce or jump close to the surface. The low angle at which these particles strike the surface can transfer momentum to smaller particles allowing them to be suspended into the atmosphere (Sehmel, 1980; Nicholson, 1988b). Depending on soil conditions, saltation can be responsible for moving 50 to 75% of surface particles. Surface creep is the rolling or sliding motion of particles, which is induced by wind stress or momentum exchanged from other moving particles. This generally applies to large particles 500 to 1000 μm in diameter and moves 5 to 25% of soil by weight (Sehmel, 1980; Nicholson, 1988b). Suspension is the process that actually ejects particles into the air. This affects particles smaller than 100 μm in diameter and moves 3 to 40% of soil by weight (Sehmel, 1980; Nicholson, 1988b).

Resuspension is often defined in terms of a resuspension factor, K, with units of m^{-1}, or a resuspension rate (Λ), with units of sec^{-1}. The resuspension factor was used in early research on reentrainment and is defined by:

$$K = \frac{C_{air}(\mu g / m^3)}{C_{soil}(\mu g / m^2)} \tag{2-8}$$

where C_{air} is the airborne concentration of a chemical species and C_{soil} is the surface soil concentration of the same species. K has significant limitations, in that it is dependent both on the height at which C_{air} is measured and the depth to which C_{soil} is measured. This factor also assumes that all airborne material is a direct result of resuspended soil-bound material, which is not the case in most situations (Sehmel, 1980; Nicholson, 1988b). Additionally, K cannot be used if soil concentrations are not uniform across the area of interest (Nicholson, 1988b).

The resuspension rate, Λ, is the fraction of a surface contaminant that is released per time and is defined by:

$$\Lambda = \frac{R(\mu g / m^2 s)}{C_{soil}(\mu g / m^2)} \tag{2-9}$$

where R is the upward resuspension flux, and Λ has units of s^{-1}. Although Λ is also dependent on the depth to which soil concentrations are measured, the resuspension rate has a number of advantages over K. Most notably, it can be applied to non-uniform areas of soil contamination, and it allows for other sources of airborne contaminants. It cannot be determined experimentally and is usually deduced by fitting results to a numerical model of airborne dispersion and deposition for the pollutant of interest (Nicholson, 1988b). Resuspension rates are dependent on many factors, including wind speed, soil moisture, particle sizes, the presence of saltating particles, and the presence of vegetation. Typical values for Λ can cover 9 orders of magnitude in the range of 10^{-12}-10^{-4} s^{-1} (Sehmel, 1980; Nicholson, 1988b).

Nicholson (1993) notes that Λ increases with increasing particle diameter because larger particles protrude faster into the turbulent air stream and the drag force increases more quickly than adhesive forces. Furthermore, in a laboratory resuspension chamber, the yields of resuspended matter decreased approximately linearly with increases in the geometric mean particle sizes of the bulk soil (Young et al., 2002). Lead is associated with the smaller size ranges in the distribution of soil particles (Van Borm et al., 1988). Young et al. (2002) suggest that this is because the higher specific surface area of small particles means that there are higher contents of organic matter or Fe/Al oxides that serve as Pb binding sites.

Saltation is a particularly important factor in determining resuspension rates. Saltation moves large quantities of soil particles and is highly efficient at ejecting particles into the airstream. Saltating particles rotate between 200 and 1000 revolutions/second and are ejected almost vertically (Sehmel, 1980). Saltating particles strike the surface at very small angles – almost horizontally – and cause an avalanching effect. In the absence of saltation, very little resuspension would occur at all (Sehmel, 1980; Nicholson, 1993). Because resuspension is driven by saltation and not the direct pick-up by wind, the size distribution of resuspended particles does not change with windspeed (Young et al., 2002).

Vehicular resuspension is the result either of shearing stress of the tires or turbulence generated by the passing vehicle (Nicholson, 1988b; Nicholson et al., 1989). This process can be particularly important, since the most contaminated roadways tend to have the most traffic. As with wind resuspension, a number of factors can affect the rate of resuspension from vehicular motion. These factors include vehicle size, vehicle speed, moisture, and particle size.

Lead in street dust appears to have a bimodal distribution. The fine mode is likely from vapor phase condensation from combustion engines, whereas the coarse mode is likely from either vehicle wear or significant coagulation of smaller particles. Al-Chalabi and Hawker (1997) observed that in roadways with significant resuspension, Pb concentrations were lower, indicating either dispersion from the source or the scavenging of smaller Pb particles by coarser particles. Abu-Allaban et al. (2003) similarly observed that Pb in road dust tended to be in the coarse mode. Measurements performed in tunnel tests indicated that $\leq$17% of Pb in PM_{10} was smaller than 2.5 μm (Lough et al., 2005).

Resuspension may occur as a series of events. Short episodes of high windspeeds, dry conditions, and other factors conducive to resuspension may dominate annual averages of upward flux (Nicholson, 1988b, 1993).

Lead concentrations in suspended soil and dust vary significantly. In suspended soils sampled near industrial emitters of Pb, PM_{10}-bound Pb varied between 0.012 and 1.2 mg Pb/ kg of bulk soil (Young et al., 2002). Tsai and Wu (1995) measured Pb in airborne particles that was 30 times higher than Pb in road dust. This enrichment factor was much higher than for other pollutants, which may indicate that Pb is more easily resuspended than other contaminants. Fractions of Pb observed in suspended dusts and soils are listed in Table 2-23.

The contribution of resuspended soil and dust to the airborne burden may be significant, particularly from highly contaminated sites. A source apportionment study in Boston indicated that soil resuspension increased the airborne concentration of Pb by as much as 0.02 $\mu g/m^3$ in the fine mode (Thurston and Spengler, 1985). Isotopic measurements in Yerevan, Armenia credited resuspension of contaminated soil with 75% of the atmospheric Pb in 1998 (Kurkjian et al., 2002). Calculations based on road dust emissions and Pb weight fractions indicate that resuspension was responsible for ~40% of overall Pb emissions to the South Coast Air Basin of California in 1989 (Lankey et al., 1998). Resuspension estimates based on modeling efforts for the same area suggest that resuspension contributed ~90% of overall Pb emissions in 2001 (Harris and Davidson, 2005). Figures 2-6 and 2-7 illustrate how air and soil concentrations may be affected by long-term resuspension.

Table 2-23. Observed Percentages of Lead in Resuspended Particulate Matter

Source	Location	Pb fraction of PM_{10} mass (%)	Pb fraction of $PM_{2.5}$ mass (%)	Reference
Paved road dust	Urban San Joaquin Valley	0.0161 ± 0.0031		Chow et al. (2003)
Paved road dust	Urban Fresno, CA	0.3 ± 0.03	0.4	Chow et al. (1994)
Paved road dust	Urban Reno and Sparks, NV		1.E-02	Gillies et al. (1999)
Paved road dust	Rural San Joaquin Valley	0.0057 ± 0.0028		Chow et al. (2003)
Unpaved road dust	Rural San Joaquin Valley	0.0058 ± 0.0073		Chow et al. (2003)
Unpaved road dust	Rural Bakersfield, CA	0.01		Chow et al. (1994)
Unpaved road dust	Residential San Joaquin Valley	0.0203 ± 0.0133		Chow et al. (2003)
Unpaved road dust	Staging area San Joaquin Valley	0.0043 ± 0.0008		Chow et al. (2003)
Agricultural soil	San Joaquin Valley	0.0063 ± 0.0059		Chow et al. (2003)
Agricultural soil	San Joaquin Valley	0.0031 ± 0.0025		Chow et al. (2003)
Agricultural soil	San Joaquin Valley	0.0062 ± 0.0034		Chow et al. (2003)
Agricultural soil	San Joaquin Valley	0.0024 ± 0.0082		Chow et al. (2003)
Agricultural soil	San Joaquin Valley	0.003 ± 0.0025		Chow et al. (2003)
Agricultural soil	Stockton, CA	0.01		Chow et al. (1994)
Playa dust	Rural Reno and Sparks, NV		1.E-03	Gillies et al. (1999)
Sand and gravel storage	Visalia, CA	0.02		Chow et al. (1994)
Construction site	Urban Reno and Sparks, NV		1.E-03	Gillies et al. (1999)

Source: Chow et al. (1994, 2003) and Gillies et al. (1999).

2.3.4 Runoff from Impervious Surfaces

The runoff of water from impervious surfaces may be a significant transport route for Pb from urban areas to soil, waterways, and catchment basins. As water runs off roadways and buildings, it can become laden with dissolved and suspended matter. Dust on roadways contains

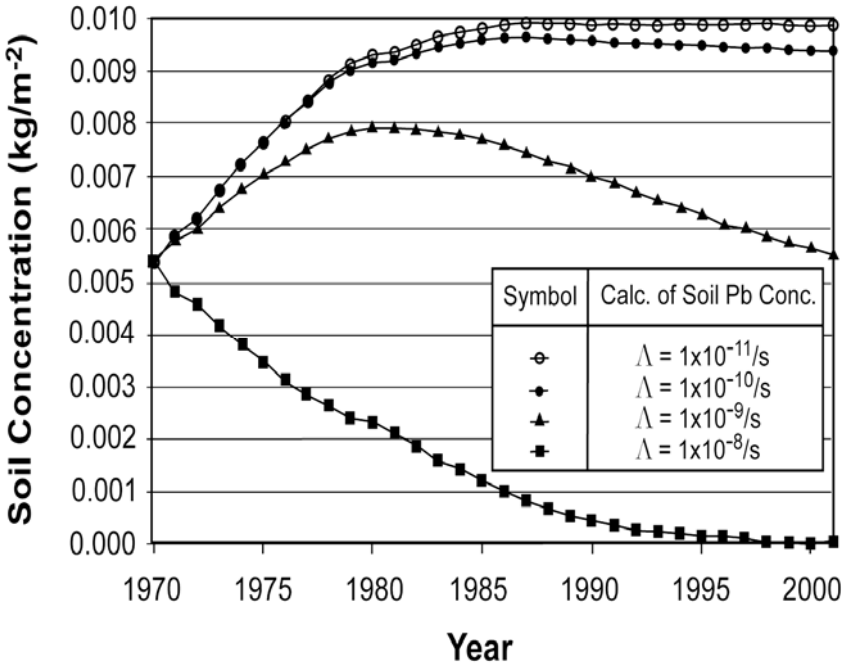

Figure 2-6. Modeled soil concentrations of lead in the South Coast Air Basin of California based on four resuspension rates.

Source: Reprinted from Harris and Davidson (2005).

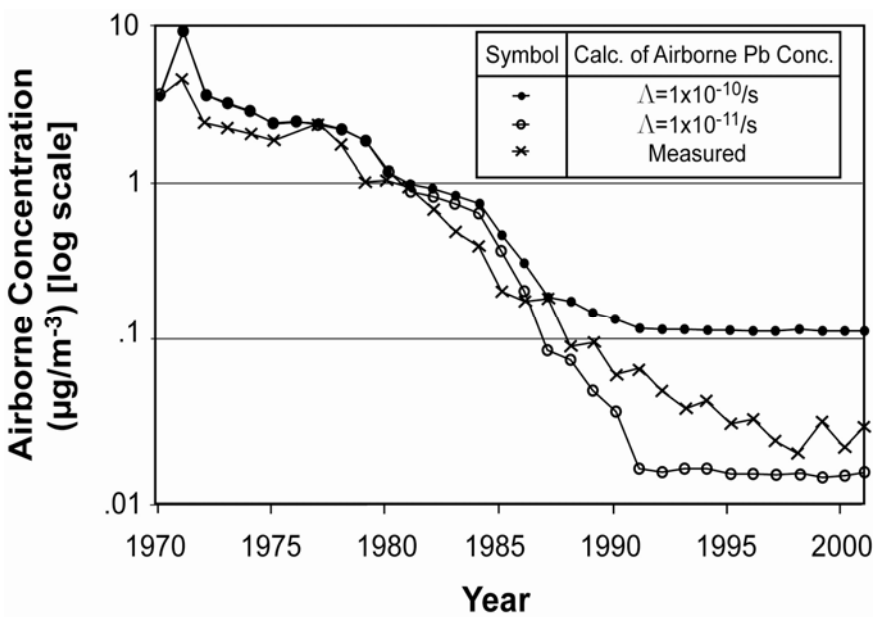

Figure 2-7. Modeled and measured airborne concentrations of lead in the South Coast Air Basin of California based on two resuspension rates.

Source: Reprinted from Harris and Davidson (2005).

a significant fraction of Pb due to vehicle wear, vehicle emissions, road wear, fluid leakage, and atmospheric deposition. Lead in road dust is discussed in further detail in Sections 2.3.3 and 3.2 of this document. Additionally, Pb-containing paints, gutters, roofing materials, and other housing materials may leach with rainfall.

Urban catchments in Fresno, CA had highly elevated soil-Pb concentrations indicative of high Pb concentrations in runoff waters (Nightengale, 1987). Basins in use since 1962, 1965, and 1969 had surface soil-Pb concentrations of 570, 670, and 1400 ppm, respectively. Nearby control soils had surface-Pb concentrations between 8.3 and 107 ppm.

Urban runoff released into a stream in State College, PA caused significant spikes in Pb concentrations (Lieb and Carline, 2000). Concentrations upstream of the release point were 1.5 µg/L. Downstream concentrations were 1.8 µg/L when there was no precipitation, and but averaged 14.6 µg/L during storm events.

The amount of Pb is removed from roadways and buildings by rainwater depends in part on the intensity of the storm. Experiments performed by Davis and Burns (1999) indicated that high intensity storms washed away significantly more exterior house paint than low-intensity storms. Other experiments showed that the amount of Pb in roadway runoff increased significantly with length of dry period prior to a rain event (Hewitt and Rashed, 1992).

Lead in runoff water primarily occurs in the particulate form, with only a relatively small fraction in the dissolved form (Hewitt and Rashed, 1992; Davis and Burns, 1999; Roger et al., 1998). Between 69% and 93% of Pb washed from painted structures was reported to be in particulate form (Davis and Burns, 1999). More than 90% of Pb in highway runoff from a rural highway in the UK was in the particulate phase (Hewitt and Rashed, 1992). Roger et al. (1998) found that Pb particles in a motorway catchment in France were typically <50 µm in diameter. Also, samples taken from road water samples in France showed that most Pb was in an inorganic, non-bioavailable form (Flores-Rodríguez et al., 1994).

Amounts of Pb from roadways vary by region, rainfall intensity, maximum inflow, rainfall duration, and antecedent dry weather periods (Shinya et al., 2000). Based on measurements taken near a roadway in France, Pb concentrations in runoff water ranged between 0.46 and 4.57 g Pb/kg of suspended PM (Roger et al., 1998). Another study of French roadways found an average Pb content of 2.36 g Pb/kg of dried material (Flores-Rodríguez et al., 1994). Thirteen storm events studied at a heavily trafficked, rural highway in England showed mean Pb

contents of 181 µg/L (Hewitt and Rashed, 1992). Of this total, 16.2 ± 6.9 µg/L was in the dissolved phase and 165 ± 101 µg/L in the particulate phase. An additional 0.36 µg/L was in an organic form. Mean Pb concentrations during four rain events studied near a roadway in Japan ranged between 17 and 39 µg/L (Shinya et al., 2000). The initial concentrations were higher, ranging from 130 to 567 µg/L. This indicates the presence of a first flush effect, in which much of the Pb contamination is removed within the initial period of rainfall. Hewitt and Rashed (1992) evident in a similar downward trend in Pb concentrations with time. However, no first flush phenomenon was evident in a study by Taebi and Droste (2004), which evaluated combined urban runoff transported to a mixed residential and commercial urban catchment in Iran. The Pb concentrations for each of 10 major rainfall events ranged between 0.018 and 0.558 µg/L. The arithmetic mean for all 10 events was 0.278 µg/L.

Studies of runoff from building materials showed high Pb concentrations from painted wood and painted brick, particularly if the paint is more than 10 years old (Davis and Burns, 1999; Davis et al., 2001). The maximum Pb concentrations were 1,900 µg/L and 28,000 µg/L associated with painted exterior wood and brick surfaces, respectively (Davis and Burns, 1999). Lead from paint is released into waters in both particulate and dissolved form. Lead concentrations observed in runoff from building surfaces are listed in Table 2-24.

Table 2-24. Lead Concentrations Observed in Runoff From Building Surfaces

Substance	Geometric Mean (µg/L)	Median (µg/L)	Mean µg/L)	Range (µg/L)	Reference
Block (painted)	9.2	8.0	38	<2-590	Davis and Burns (1999)
Brick (painted)	22	16	580	<2-28000	Davis and Burns (1999)
Wood (painted)	43	49	170	<2-1900	Davis and Burns (1999)
0-5 yr. old paint	8.0	8.1	27	<2-370	Davis and Burns (1999)
5-10 yr. old paint	18	14	120	<2-2600	Davis and Burns (1999)
>10 yr. old paint	81	88	810	<2-28000	Davis and Burns (1999)
Roofs	6.0	5.2	38	<2-590	Davis and Burns (1999)
Residential roofs		2	1.5		Davis et al. (2001)
Commercial roofs		12	62		Davis et al. (2001)
Institutional roofs		64	64		Davis et al. (2001)

Source: Davis and Burns (1999) and Davis et al. (2001).

Matthes et al. (2002) studied runoff from lead sheet to simulate lead in gutters, roofs, piping, siding, and sculptures. Typical concentrations in runoff ranged between 700 and 3700 mg/L. This was attributed to the solubility of cerrusite (Pb carbonate) and hydrocerrusite (Pb hydroxy carbonate), which form on the surface of air-exposed Pb. Lead corrosion (cerrusite and hydrocerrusite) dissolution rates from Pb sheets were measured at 14.3 to 19.6 millimoles of lead/m^2 per year (Matthes et al., 2002).

The amount of Pb removed by runoff events varies. Hewitt and Rashed (1992) estimate that ~8% of Pb and 5% of organic Pb emitted from vehicles is removed by highway drainage waters. Shinya et al. (2000) estimate that total Pb loads for a roadway in Japan prior to four storm events ranged between 0.053 and 0.771 mg Pb/m^2. These storm events removed half of the load in 0.07 to 3.18 hours after the start of the rainfall event.

Davis et al. (2001) estimate the total annual loading of Pb from all sources to be between 0.069 and 0.18 kg Pb/ha. They estimate that 80 to 90% of this is derived from runoff from buildings.

2.3.5 Leaching of Soil Lead

Although Pb is generally relatively immobile and has a long retention in most soils, soil Pb has some capacity to leach through the soil column, potentially contaminating ground water. Lead sorbs strongly to constituents of the soil matrix and is only weakly soluble in pore water, so the leaching of Pb is a much slower process than the leaching of many other contaminants (Marcos et al., 2002; Zhang and Xu, 2003; Ünlü, 1998; Pang et al., 2002). The sorbing capacity of the soil and the solubility of the contaminants can be affected by the hydraulic conductivity of the soil, the composition of the soil solution, the content of the soil organic matter, the content of the soil clay minerals, soil pH, microbial activity, preferential flow through plant root channels and animal holes, and geochemical reactions (Rhue et al., 1992; Elzahabi and Yong, 2001). The experiments of Erel et al. (1997) on soil columns indicate that anthropogenic Pb is more readily available for leaching than Pb that naturally occurs in the soil.

Lead can bind to many different surfaces in the heterogeneous soil matrix. This adsorption greatly affects mobility and is dependent on the characteristics of the soil and Pb compounds. Lead is partitioned between the soil water solution, precipitated forms, secondary Fe or Mn oxides, carbonates, organic matter, sulfides, or the surfaces of clay, humus, or silicate

particles (Badawy et al., 2002; Venditti et al., 2000; Cajuste et al., 2000; Erel and Patterson, 1994). The most labile fraction of Pb is adsorbed to the surfaces of colloid soil particles, which may include organic matter, clay, oxides, or carbonates (Erel et al., 1997). Lead leached from a limestone soil during a sequential fractionation procedure was exclusively in the iron/manganese oxide form (Hee, 1994). A study of industrially contaminated soils found that ~50% to 60% of the Pb was not susceptible to leaching during any phase of a sequential fractionation procedure (Cajuste et al., 2000). The remaining Pb was found primarily in the carbonate and Fe-Mn oxide fractions, with sizeable amounts in the organic and exchangeable phases. None of the Pb was water soluble. Maskall and Thornton (1998) also observed a high fraction of Pb in the carbonate form in highly contaminated soil. The unusual presence of carbonate-bound Pb is probably due to the formation of cerrusite ($PbCO_3$) in soils contaminated with calcareous slag wastes (Maskall and Thornton, 1998). Lead migration in this contaminated soil was associated with Fe-Mn oxides. A third contaminated site was tested by Jing et al. (2004). These soils showed 57% of Pb in the Fe-Mn oxide form, 29% in the carbonate form, and just 5% in the residual, soil-bound form.

High chlorine content in soil has been shown to increase Pb leaching (Ünlü, 1998). Chloride complexation with Pb enhances lead solubility.

The pore-water velocity is inversely proportional to sorption rates. At low flow, the longer retention times Pb to more complete sorption of Pb to soil particles (Pang et al., 2002).

In laboratory experiments on soil columns, transport of Pb was enhanced by the introduction of soil colloid suspensions (Karathanasis, 2000). Colloids increased transport of not only colloid-bound Pb but also dissolved Pb. Colloid transport was enhanced by increasing the colloid surface charge, increasing the pH, increasing the amount of organic carbon, increasing the soil macroposity, decreasing the colloid size, and decreasing the Al, Fe, and quartz contents (Karathanasis, 2000). Colloid binding and co-transport of Pb are important mechanisms for Pb migration, but colloids also enhance the flow of Pb through physical blockage from exchange sites, competitive sorption, and organic complexation (Karathanasis, 2000). Denaix et al. (2001) observed that most of the Pb-transporting colloids in an acidic, loamy soil were biological in nature. The Pb concentration in the colloid fraction was not correlated with pH, colloidal organic carbon contents, or colloidal silicon concentrations (Denaix et al., 2001). Approximately 50% of the total Pb transfer in these experiments was attributed to colloidal transfer.

At low pH, metal species bound to carbonates, hydroxides, and other soil matrix components are more likely to dissolve into solution (Maskall and Thornton, 1998; Elzahabi and Yong, 2001; Badawy et al., 2002). This increases the rate of Pb migration through the soil. The experiments of Jing et al. (2004), which followed eight different leaching protocols, suggest that pH is the primary factor in determining the concentration of Pb in leached solution. At pH >12, Pb forms soluble hydroxide anion complexes and leaches out of the soil column. At pH between 6 and 12, Pb leachibility is low due to adsorption and precipitation. At pH <6 free Pb ions leach into the pore water and are removed from the soil columns. Rhue et al. (1992) observed that organic Pb species (Me_2Pb^{2+} and Et_2Pb^{2+}) were best absorbed at pH 6.2 and 7.2, respectively. Sorption decreased at pH <5 and >8.2 (Rhue et al., 1992).

A partition coefficient, K_d, is often used to describe the susceptibility of Pb to leaching. This value is used to compare the fractionation of a contaminant between liquid and solid forms. K_d is defined by the following equation:

$$K_d = S/C' \tag{2-10}$$

where S is the total concentration of Pb adsorbed in the solid phase and C' is the concentration of Pb in pore water solution (Elzahabi and Yong, 2001). K_d increases with increasing pH (up to 7.0) and increasing distance from the leachate source (Elzahabi and Yong, 2001; Sheppard and Sheppard, 1991). K_d decreases with an increase in the influent heavy metal concentration and the degree of saturation (Elzahabi and Yong, 2001). The highest value of K_d appears to be near the source of Pb contamination. Values of K_d in the literature cover many orders of magnitude between 1.20 L/kg and "infinity" (when no Pb can be detected in pore water). These values are listed in Table 2-25. For more information on Pb solid-solution partitioning see Chapter 7.

The rate of migration through the soil has been estimated in many different studies. Using Pb isotopes, Erel et al. (1997) estimate the rate of Pb migration to be 0.5 cm/year in soils collected from rural locations in Israel. Sheppard and Sheppard (1991) measured the rate of flow through spiked soils, which were highly acidic and had a low organic matter content. These soils, which were especially susceptible to leaching, exhibited migration rates of 0.3 cm/day during the first year of experiments. The migration rate appeared to slow down in subsequent years. Cores taken at smelting sites used during the Roman era, medieval times, and the

Table 2-25. Soil/Water Partition Coefficients for Several Different Soils and Conditions

Kd (L/kg)	pH	Beginning Soil Water Content (%)	Soil Type	Reference
12.68	4.0	26.69	Illitic (spiked)	Elzahabi and Yong (2001)
3.23	4.0	28.20	Illitic (spiked)	Elzahabi and Yong (2001)
1.20	3.5	26.29	Illitic (spiked)	Elzahabi and Yong (2001)
1.36	3.5	26.32	Illitic (spiked)	Elzahabi and Yong (2001)
~6000	n.a.	n.a.	Brown pseudopodzolic	Alumaa et al. (2002)
~3000	n.a.	n.a.	Rendzina	Alumaa et al. (2002)
~5000	n.a.	n.a.	Gley podzolic	Alumaa et al. (2002)
20	4.9	n.a.	Acidic (low-organic-matter sand)	Sheppard and Sheppard (1991)
9000	4.8	n.a.	Sphagnum peat	Sheppard and Sheppard (1991)
92.99	4.45	n.a.	Mining site	Merrington and Alloway (1994)
14.25	4.45	n.a.	Mining site	Merrington and Alloway (1994)
125.58	5.01	n.a.	Mining site	Merrington and Alloway (1994)
95.51	5.01	n.a.	Mining site	Merrington and Alloway (1994)
1330 ± 200	3.0-4.0	n.a.	Acidic (high-organic-matter peat)	Deiss et al. (2004)

Source: Elzahabi and Yong (2001), Alumaa et al. (2002), Sheppard and Sheppard (1991), Merrington and Alloway (1994), and Deiss et al. (2004).

18th century underwent sequential extraction (Maskall and Thornton, 1998). The estimated Pb migration rates at the Roman, medieval, and 18th century sites were 0.07 to 0.54 cm/year, 0.31 to 1.44 cm/year, and 0.11 to 1.48 cm/year, respectively. Using Pb measurements from soil cores in agricultural fields, Zhang (2003) estimated a maximum deposition flux of approximately 27 (±14) $\mu g/cm^2$-year (270 mg/m^2-year) to have occurred around 1940 (attributed by the author to residential coal burning). Measurements made for more recent time periods were lower, but accompanied by such variability as to be indistinguishable from zero via the study methodology in more recent years. Over the 50-year period from 1950-2000, the authors estimated a loss from surface soils (to lower horizon soils) of more than 50% of atmospherically-derived Pb.

Mass balance calculations of Miller and Friedland (1994) suggest that the response time (or soil horizon flushing time) for Pb is 17 and 77 years in the organic horizons of northern hardwood and spruce-fir forests, respectively. Similar calculations by Kaste et al. (2003) at the same site predicted that anthropogenic Pb will take ~60 and ~150 years to be transported through the organic horizon in the deciduous and spruce-fir forests, respectively. The difference in response times for the two forests may be due to differences in the litter depth and/or in the rate of litter decomposition.

Soil tested from a car battery salvage facility showed a significantly greater Pb concentration in the leached solution than in a reference soil (Jensen et al., 2000). Lead concentrations in the leached solution went as high as 8000 μg/L. Other industrially contaminated soils did not show such high rates of leaching, but these other soils had nearly neutral pHs.

Isotopic ratios in soil cores in the Sierra Nevada, California showed that 21% of Pb at a depth of 30 cm had anthropogenic origins and had migrated from the surface (Erel and Patterson, 1994). The remaining 79% of Pb at this depth was naturally occurring.

Physical mixing of soils through animal activity may also increase the rate of Pb migration. Mace et al. (1997) observed a significant decrease in Pb transport time through soil as a result of rodent activity in a southern California location.

Vilomet et al. (2003) used isotopes to trace the leaching of Pb from a landfill into groundwater in France. The active landfill has been in use since 1900 and has no bottom liner. Detectable quantities of leached Pb were seen as far as 4600 m downgradient (Vilomet et al., 2003).

2.3.6 Transport in Aquatic Systems

Chemical, biological, and mechanical processes govern the cycling of Pb in aquatic environments. The main focus here is on the exchange between sediment and surface water, which is affected by many different factors including salinity, the formation of organic complexes, redox conditions, and pH (Arakel and Hongjun, 1992).

Lead enters surface waters from a number of sources. Atmospheric deposition is the largest source, but urban runoff and industrial discharge are also significant (Peltier et al., 2003;

Hagner, 2002; Perkins et al., 2000). As expected, concentrations in surface waters are highest near sources of pollution.

The dispersal of Pb in waterways is relatively quick. If Pb is emitted into waterways as a point source, water concentrations decrease rapidly downstream of the source (Rhoads and Cahill, 1999; Hagner, 2002; Kurkjian et al., 2004; Peltier et al., 2003). Lead is removed from the water column through flushing, evaporation, or sedimentation (Schell and Barnes, 1986). Kurkjian et al. (2004) noted that first order approximations of concentrations of non-conservative pollutants (such as Pb) can be made by using the exponential decay curve:

$$C = C_o e^{kx} \tag{2-11}$$

where C is the pollutant concentration, C_o is the concentration at the source, x is the downstream distance from the source, and k is the decay rate in km^1. For the Debed River in Armenia, Kurkjian et al. (2004) found that a decay rate of 0.57 km^1 provided the best fit to measured Pb concentrations.

Metals in waterways are transported primarily as soluble chelates and ions, constituents of PM, or by adsorption onto suspended organic or inorganic colloids (Arakel and Hongjun, 1992). The last two are the most important for Pb. The predominant chemical forms of Pb that interact with aqueous ecosystems are PbO and $PbCO_3$ (Schell and Barnes, 1986). Lead is adsorbed on colloids that are typically secondary clay minerals, Fe-Mn oxides or hydroxides, or organic compounds (Arakel and Hongjun, 1992). The concentration of Pb appears to increase with increasing salinity (Arakel and Hongjun, 1992).

Schell and Barnes (1986) describe water columns as "transient reservoirs" for pollutants. They found mean residence times for Pb in two lakes and a reservoir to be between 77 and 250 days, although it should be noted that residence times tend to be shorter in turbulent waterways. Lead concentrations in water are attenuated by the presence of $Al(OH)_3$ precipitation, which is responsible for an estimated 54% of total Pb loss, and by the adsorption of Pb onto other particles which settle out of the water column, which makes up the other 46% of Pb loss (Kurkjian et al., 2004). Schell and Barnes (1986) measured sedimentation rates for anthropogenic Pb, which ranged between 0.036 g cm^2 a^1 and 0.064 g cm^2 a^1.

Concentrations of Pb in sediments roughly follow the concentrations of Pb in overlying water (Kurkjian et al., 2004; Rhoads and Cahill, 1999). Thus, Pb concentrations in sediment are highest near sources and decrease downstream.

Lead preferentially sorbs onto small particles rather than large particles. Small grain sizes and the larger surface area per unit weight Pb to greater potential for adsorption (Rhoads and Cahill, 1999). Concentrations of metals increase approximately logarithmically with decreasing particle size.

Organic matter in sediment has a high capacity to accumulate trace elements. High humic levels may Pb to greater Pb contamination in sediments (Rhoads and Cahill, 1999; Kiratli and Ergin, 1996).

Sulfides are another potential source of Pb adsorption. This is especially true under anoxic conditions (Kiratli and Ergin, 1996; Perkins et al., 2000). An increase in the amount of sulfide in pore water was shown to decrease the dissolved concentration of Pb (Peltier et al., 2003).

Lead in sediment can also be sequestered on iron or manganese oxides (Peltier et al., 2003; Gallon et al., 2004; Schintu et al., 1991). These forms may make Pb more susceptible to recycling into the overlying water column (Schintu et al., 1991).

Lead appears to be relatively stable in sediment. It has a very long residence time, and many studies suggest that Pb is not mobile in the sediment. However, many other studies suggest that Pb-containing particles can be remobilized into the water column (Ritson et al., 1999; Steding et al., 2000; Hlavay et al., 2001; Kurkjian et al., 2004; Peltier et al., 2003; Gallon et al., 2004). For example, Steding et al. (2000) observe that isotopic concentrations of Pb in the San Francisco Bay match those of leaded gasoline from the 1960s and 1970s, suggesting that recontamination by sediment may be a significant source of Pb to overlying waters. Ritson et al. (1999) similarly observed that there was a negligible reduction in Pb concentrations in the San Francisco Bay despite the closing of a nearby Pb smelter, the implementation of municipal effluent controls, and the elimination of Pb additives to gasoline. That concentrations have remained high may suggest recycling of sediment Pb. Similarly, in a study of water Pb concentrations in the North Sea, Pb concentrations did not decrease significantly with the elimination of major sources (Hagner, 2002). This also may indicate continued high rates of atmospheric deposition or cycling of Pb stored temporarily in sediment.

Modeling efforts of Gallon et al. (2004) indicate that processes that resuspend sediment (such as diffusion, bioturbation, and bioirrigation) are small compared to sedimentation of colloidal particles. Kurkjian et al. (2004) suggest a correction factor for equation (2-11) to account for the contribution of Pb from sediment.

$$C = C_o e^{(-kx)} + I_s \qquad (2\text{-}12)$$

where I_s is the amount of Pb resuspended into the water column. Depending on the region of the river under discussion, the authors extrapolated I_s values in the range of 1.3-2.8 µg Pb/L.

2.3.7 Plant Uptake

Plants that take up Pb can be a source of Pb exposure for wildlife, livestock, and humans that consume contaminated plants. More thorough discussion of soil Pb extraction by plants and subsequent effects on ecosystem health can be found in Chapter 7 of this document.

Plants grown in soils contaminated by mine spoils (e.g., Cobb et al., 2000), smelting operations (e.g., Barcan et al., 1998), sludge amendments (e.g., Dudka and Miller, 1999), contaminated irrigation water (e.g., Al-Subu et al., 2003), or Pb-containing agrochemicals (e.g., Azimi et al., 2004) have higher than natural concentrations of Pb. In general, higher Pb concentrations in soils typically result in increased Pb levels in plants.

Although the transfer of soil Pb to plants and direct stomatal uptake of atmospherically deposited Pb are generally small, all plants can accumulate Pb to some degree (Finster et al., 2004). The rate of uptake is affected by plant species, soil conditions, and Pb species.

Of all the factors affecting root uptake, pH is believed to have the strongest effect (Dudka and Miller, 1999). Acidic soils are more likely to have Pb in solution and therefore available for absorption. This is sometimes attenuated by liming.

Most Pb in plants is stored in roots, and very little is stored in fruits (e.g., Finster et al., 2004; Cobb et al., 2000). Of 33 edible plants grown in urban gardens, roots had a median Pb concentration that was 12% of the soil-Pb concentration (Finster et al., 2004). Shoot Pb, when detectable, was just 27% of root Pb. Root vegetables seem the most prone to Pb uptake, followed by leafy vegetables (Dudka and Miller, 1999; Finster et al., 2004). Fruits and grains do not seem as susceptible to Pb contamination.

Metals that are applied to soil as salts (usually as sulfate, chloride, or nitrate salt) are accumulated more readily than the same quantity of metal added via sewage sludge, flue dust, or fly ash (Dudka and Miller, 1999). This is likely because metal salts Pb to the formation of metal chloride complexes and ion pairs, which can increase metal diffusion and subsequent root uptake.

2.3.8 Routes of Exposure for Livestock and Wildlife

There are many routes of Pb exposure for livestock and wildlife, including food ingestion, drinking water, and inhalation for terrestrial organisms. For aquatic organisms, the main routes of Pb exposure are food ingestion and water intake. A few representative studies which have analyzed routes of Pb exposure for livestock and wildlife are summarized here. For a discussion of health effects, toxicity, and Pb concentrations in animal tissue, see Chapter 7.

Lead concentration of plants ingested by animals is primarily a result of atmospheric deposition of Pb particles onto plant surfaces rather than the uptake of soil Pb through plant roots (Steinnes, 2001; Palacios et al., 2002; Dudka and Miller, 1999). The uptake of Pb by the lowest trophic levels – invertebrates, phytoplankton, and krill for example – are some of the most important avenues for introducing Pb into food chains (Pilgrim and Hughes, 1994; Sanchez-Hernandez, 2000; Hagner, 2002).

Some of the highest levels of Pb exposure in animals occur near major sources like smelters. In two studies of horses living near smelters, the estimated ingestion rate was in the range of 2.4 to 99.5 mg Pb/kg body weight per day (Palacios et al., 2002) and 6.0 mg Pb/kg body weight per day (Liu, 2003). Both exposure rates were well above the estimated fatal dose for horses. Sheep grazing near smelters were similarly poisoned (Liu, 2003; Pilgrim and Hughes, 1994). Installation of pollution controls at a Pb smelter in Slovenia greatly reduced the amount of Pb in nearby vegetation and the blood-Pb levels of cows grazing on this vegetation (Zadnik, 2004). However, Pb concentrations in topsoil at this site have not noticeably declined in the 20 years since the pollution controls were implemented.

The amount of Pb entering the food chain depends highly on the species of the animal, the species of their food, and where the organisms live. A study of sheep living in the southernmost part of Norway (the most polluted part of the country), showed a strong correlation between liver Pb concentrations and moss concentrations (Steinnes, 2001). The sheep fed almost exclusively

on a grass that easily picks up atmospherically deposited Pb. Correspondingly high Pb levels were also observed in hare and black grouse in this region. Similarly, a study of Pb concentrations in raccoon tissues showed much higher Pb levels in urban raccoons than in rural raccoons (Khan et al., 1995). This may be because urban raccoons are exposed to higher air concentrations, ingest human refuse, or frequently visit storm sewers. In general, ruminant animals appear to be more resistant to Pb ingestion than monogastric animals (Humphreys, 1991).

Acute Pb poisoning observed in Laysan albatross (*Phoebastria immutabilis*) chicks was traced to the direct ingestion of paint chips by using isotopic analysis (Finkelstein et al., 2003). Blood-Pb concentrations in *P. immutabilis* at the Midway Island National Wildlife Refuge had a geometric mean of 190 µg/dL. *P. immutabilis* chicks at a reference site had blood-Pb levels of 4.5 µg/dL. Lead levels are somewhat elevated even in Antarctic animals (Sanchez-Hernandez, 2000). Antarctic food systems are supported by krill (*Euphausia superba*), which is the primary food source for organisms in higher trophic levels. Lead concentrations measured in *E. superba* were in the range of 0.17-12.0 ppm by dry weight. This is probably elevated above natural levels due to anthropogenic Pb inputs (Sanchez-Hernandez, 2000).

Lead contamination in mammals and fish livers was shown to be higher in highly polluted coastal zones than in the open sea (Hagner, 2002). In foraminifers, which are meiobenthic organisms, high sediment Pb concentrations corresponded to high tissue Pb concentrations. Sediment concentrations were 10 to 20 times higher than foraminifer concentrations. Fish take in Pb either in their food or in water through their gills. The relative importance of these two mechanisms depends largely on the fish species. A literature survey suggests that there has been no observable decrease in fish muscle and liver concentrations of Pb over the past twenty years in marine or freshwater environments (Hagner, 2002). Lead concentrations in the harbor porpoise (*Phocoena phocoena*) appear to increase with the age of the animal, but this was not evident for the common seal (*Phoca vitulina*) (Hagner, 2002). Shrimp (*Palaemonetes varians*) were shown to absorb 4 to 8% of the Pb content of its prey (Boisson et al., 2003). Between 52% and 57% of the Pb accumulated from food was irreversibly retained in *P. varians* tissue. Just 2% of dissolved Pb accumulated from water was retained in tissue (Boisson et al., 2003).

2.4 METHODS FOR MEASURING ENVIRONMENTAL LEAD

The previous 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986) contained a detailed review of sampling and analytical methods for measuring Pb in environmental media. Included in that document were discussions of site selection criteria, sampling methods, sample preparation, and analysis techniques. Furthermore, the document included discussion of sampling of Pb emissions from mobile and stationary sources. In this section, only a very brief summary is provided for approaches for sampling and analysis of Pb in environmental media. For a more comprehensive discussion, the reader is referred to the 1986 Lead AQCD.

Emissions can be estimated from measurements at sources using grab samples, periodic samples, or continuous monitoring. Determining the rate of emissions requires knowing both the fluid flow rate and the concentration of Pb in the fluid, usually air or water. Thus, it is much easier to measure emissions from stacks than it is to measure fugitive, diffuse, or nonpoint emissions (Frey and Small, 2003). Much of the recent improvement in the measurement of Pb emissions from sources is due to better sampling and analytical equipment. For example, better dilution tunnels can provide reliable samples from in-stack sampling, and improved analytical methods (such as inductively coupled plasma mass spectrometry) permit determination of Pb at much lower levels than in years past. This means that it is possible to obtain data from short sampling runs, permitting better time resolution.

Wet deposition can be collected using precipitation buckets that seal tightly immediately before and after rain. Dry deposition on land can be sampled using surrogate surfaces, such as Teflon plates (Davidson et al., 1985; Davidson and Wu, 1990); or it can be done alternatively by leaf-washing (Lindberg and Lovett, 1985) or by sampling throughfall precipitation that washes previously deposited Pb off the vegetation and onto the forest floor (Wu et al., 1992b). Dry deposition onto bodies of water is more difficult to estimate, usually requiring airborne concentrations used in conjunction with deposition velocity estimates (Zufall and Davidson, 1997). Subsequent analysis of all of these samples can be performed by atomic absorption spectrometry, neutron activation analysis, x-ray fluorescence, or proton-induced x-ray emission (Koutrakis and Sioutas, 1996), or by inductively-coupled plasma mass spectrometry (ICP-MS) (U.S. Environmental Protection Agency, 1991).

Recently developed single-particle instruments can identify which particles contain Pb, and what other elements are present in the same particle. Such instrumentation can also provide

information on the size of the particle (Pekney et al., 2006; Silva and Prather, 1997). Although such instruments are not able to determine the precise mass of Pb in each particle, they can provide valuable data on the characteristics of particles that contain Pb from individual sources or source categories. Such "fingerprinting" methods can be used to identify sources of Pb-containing particles in the environment.

2.5 SUMMARY

This chapter discusses sources of airborne Pb that generally fall in three categories: natural sources, stationary sources, and mobile sources.

Nationwide, U.S. airborne Pb emissions fell 98% between 1970 and 2003 (U.S. Environmental Protection Agency, 2003). The elimination of alkyllead additives to automotive gasoline was principally responsible for the drop, although air-Pb emissions fell by 5% from 1993 to 2002 after the total phaseout of leaded fuel (U.S. Environmental Protection Agency, 2003). Figure 2-8 shows the decline in estimated U.S. Pb emissions from 1982 through 2002.

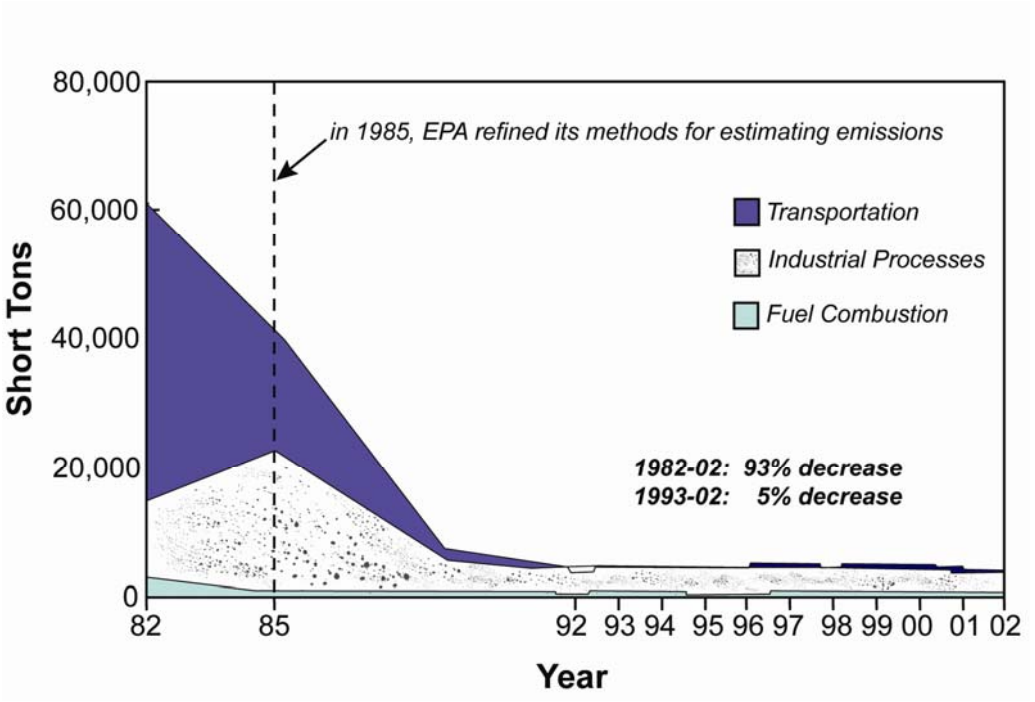

Figure 2-8. Trends in U.S. air lead emissions, 1982-2002.

Source: U.S. Environmental Protection Agency (2003).

For most of the past 50 to 60 years, the primary use of Pb was as additives for gasoline. Leaded gasoline use peaked in the 1970s, and worldwide consumption has declined since (Nriagu, 1990). The largest source of air-Pb emissions was leaded gasoline throughout the 1970s and 1980s. In 1980, on-road vehicles were responsible for ~80% of air-Pb emissions, whereas in 2002, on-road vehicles contributed less than half of a percent (U.S. Environmental Protection Agency, 2003). In every case where the U.S. Pb NAAQS has been exceeded since 2002, stationary point sources were responsible (www.epa.gov/air/oaqps/greenbk/inte.html).

Pirrone and Keeler (1996) estimated that motor vehicles were the source of 85% of total Pb deposition to the Rouge River watershed in 1982, but only 2.1% of Pb deposition flux in 1992; in 1992, the major sources of Pb deposition flux were estimated to be steel (36%), municipal solid waste combustion (29%), sewage sludge incineration (21%) and coal combustion (10.4%). Source apportionment analyses in $PM_{2.5}$ samples collected in 1998-1999 in the New York – New Jersey Harbor Bight area indicated that fossil fuel combustion, oil combustion, metal processing industry, and waste incineration were the major sources of Pb in fine particles (Gao et al., 2002).

Stationary sources emitted an estimated 1,662,000 kilograms nationwide in 2000 (U.S. Environmental Protection Agency, 2003). The largest emitters are now in the industrial sector, which includes iron and steel foundries, smelters, combustion of hazardous and solid waste, and others (Harris and Davidson, 2005). These emissions are not confined to the air—~90 facilities nationwide also generate 90% of the Pb-containing solid hazardous waste (Chadha et al., 1998). Nationwide air emissions in 2000 were estimated as 1,600 tons in total emissions of Pb, with contributions of 288 tons from iron and steel foundries, 234 tons from industrial boilers/heaters, 165 tons from coal-fired utility boilers, and 143 tons from mobile sources (see Table 2-8).

It should be noted that Pb emission inventories have significant omissions and discrepancies (Harris et al., 2006; Chadha et al., 1998). An analysis of four emission inventories for Pb in southern California showed that major Pb emitters were missed by all four databases, and that the databases were not consistent with one another nor updated regularly (Harris et al., 2006). Thus, the data noted above are probably a lower limit for Pb emissions. Efforts to develop accurate databases of Pb emissions are needed.

Natural processes contribute only a relatively small amount to the overall load of Pb in the environment. Nriagu and Pacyna (1988) estimate mean global natural emissions are at least an

order of magnitude smaller than anthropogenic emissions. Natural sources include volcanoes, seasalt spray, wild forest fires, wind-borne soil particles, and biogenic processes (Nriagu, 1989).

Air is the major transport route for Pb emissions. Deposition of airborne pollutants to surfaces has been observed in the most remote places on Earth, including the Arctic and Antarctic. Mass balance calculations performed on an agricultural plot in France indicate that atmospheric deposition is the dominant source of Pb to soil even when Pb-containing fertilizer is applied (Azimi et al., 2004). However, on a local scale, solid waste disposal or mine tailings may be the predominant source of soil Pb.

A rigorous comparison of resuspension, leaching, and plant uptake "removal" rates for soil Pb has not been undertaken. Resuspension of Pb-containing particles is likely the dominant removal mechanism from surface soil when soil pH is high. Leaching may dominate when soil pH is low. Leaching of Pb through soil occurs more rapidly than uptake to pea or wheat crops (Azimi et al., 2004). More research is needed to compare removal rates for other plants with soil Pb migration and resuspension rates.

Surface waters are contaminated through several routes. On a global scale, sediment resuspension and wet and dry deposition are the predominant contributors to Pb concentrations in surface water. On a local scale, industrial effluent and urban runoff may dominate.

The major routes of Pb transport into the food chain appear to be the ingestion of contaminated plants, ingestion of contaminated water, and inhalation of contaminated air. Research into the relative importance of each of these transport routes is needed.

Measurements conducted in essentially any ecosystem worldwide show some level of Pb contamination. Anthropogenic Pb reaches these ecosystems through many possible transport routes, such as those shown in Figure 2-9.

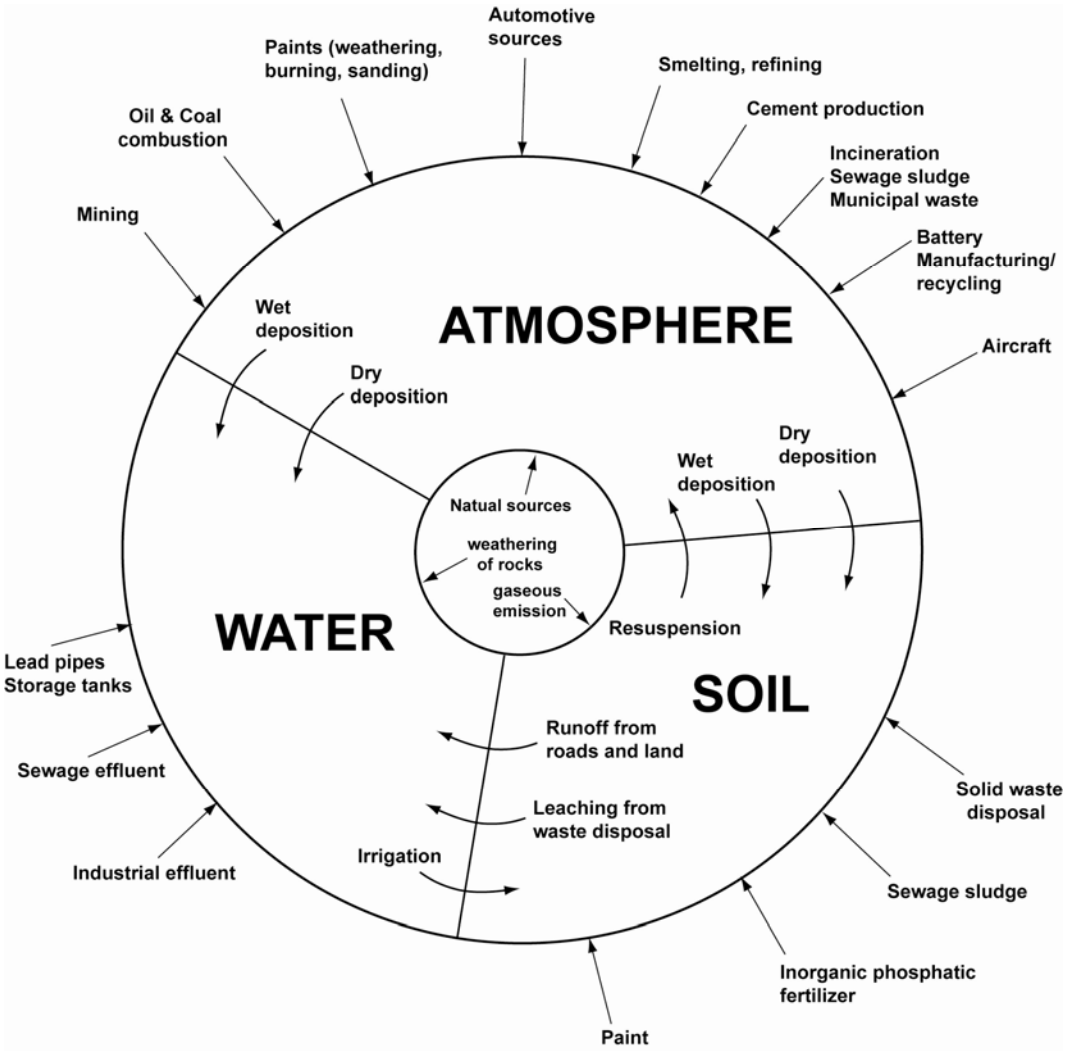

Figure 2-9. Transport pathways for lead in the environment.

Source: Modified from Zabel (1993).

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3. ROUTES OF HUMAN EXPOSURE TO LEAD AND OBSERVED ENVIRONMENTAL CONCENTRATIONS

Introduction

This chapter assesses information regarding human exposures to lead (Pb) via various media and routes of exposure. Lead has been observed in measurable quantities in nearly every environmental medium all over the world. As summarized in the 1986 Lead Air Quality Criteria Document (1986 Lead AQCD), human exposure to Pb occurs through various routes, as shown in Figure 3-1. That figure is a simplified diagram of multimedia routes of exposure via various environmental media, with a focus on the ambient air. The multimedia aspects of Pb exposure can be seen in that Pb emissions to the air contribute to Pb concentrations in water, soil and dusts; and Pb in soil and dust also can make important contributions to Pb concentrations in ambient air. The relative contributions of Pb from different media and different sources to human Pb exposure depend on factors such as the proximity of major sources to the residence and workplace of the individual, the condition of the residence (especially the presence and condition of any Pb-based paint present) and whether the residence is in an urban, suburban or rural location.

In general, Pb exposure in the United States has fallen with the elimination of leaded gasoline, Pb-based paint and Pb solder in cans. As airborne concentrations of Pb have fallen in the United States, a corresponding decrease in blood-Pb levels of the U.S. population has occurred. In a meta-analysis of 19 studies from six continents, a strong linear correlation was observed between blood-Pb levels and gasoline Pb levels (Thomas et al., 1999). As gasoline Pb was reduced to zero in the study countries, airborne Pb concentrations declined and converged to less than 0.2 $\mu g/m^3$, and blood Pb levels also declined, converging to a median of 3 µg/dL. However, the potential for high Pb exposures remains, particularly in areas near major Pb sources or with exposures to Pb-based paint or high Pb levels in drinking water.

This chapter discusses the evidence related to current concentrations of Pb in different media and human Pb exposure contributions from various media. The chapter first focuses on exposure to Pb from ambient air, briefly summarizing information available from various U.S.

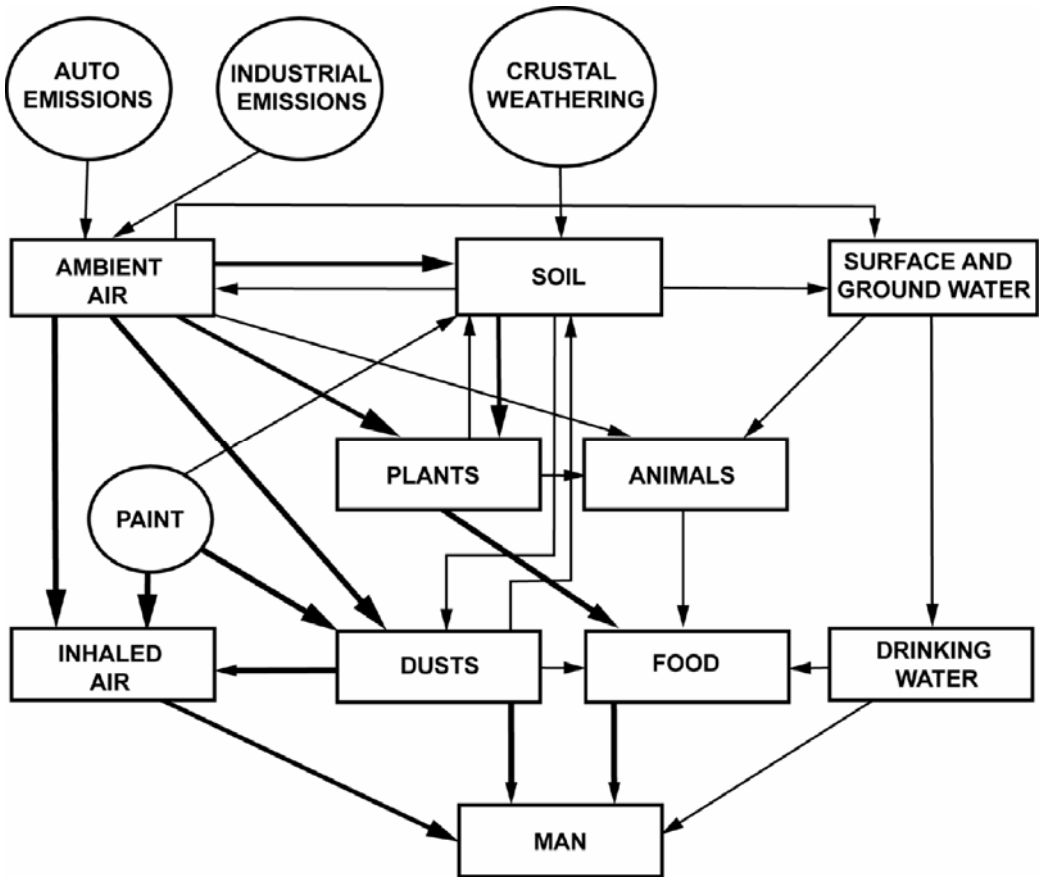


Figure 3-1. Principal pathways of lead from the environment to humans. Heavy arrows are those pathways discussed in greatest detail in this chapter.

Source: Modified from 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986).

ambient air monitoring networks. The chapter also assesses information related to Pb exposure from soil or dust, drinking water, dietary intake of foods or beverages, Pb-based paint, and from various other sources. In each section, Pb concentrations reported for these various media are discussed, along with available evidence on the contribution of Pb in multiple media to human Pb exposures. As discussed in more detail in Chapters 4 and 6, concentrations of Pb in blood and bone are the most common indices used as biological indicators or biomarkers of human Pb exposure.

3.1 EXPOSURE: AIR

Widespread emissions from anthropogenic sources (described in Chapter 2) have contributed to elevated environmental Pb concentrations. In fact, airborne Pb concentrations in many places throughout the world increased several orders of magnitude during the past seventy years, largely due to the use of leaded gasoline additives (Miller and Friedland, 1994). The lowest Pb concentrations measured are at the South Pole, where an average concentration of 0.076 ng/m^3 was recorded (Maenhaut et al., 1979). Even at this remote location, it is likely that the airborne Pb levels have exceeded historical background levels. This is evidenced by Pb concentrations in Arctic ice sheets that increased from <1 ng/kg in 800 BC to 200 ng/kg in the 1960's (Murozumi et al., 1969).

Airborne concentrations of Pb in the United States have fallen dramatically over the last 30 years due largely to the phase out of leaded gasoline additives. Figure 3-2 shows the sharply declining trend in overall U.S. airborne Pb concentrations since 1983. Major declines over several orders of magnitude have been observed not only in urban areas, but also in rural regions and remote locations. Data taken at rural sites throughout the United States since 1979 showed a similar decline (Eldred and Cahill, 1994).

The United States has not been the only country to experience a significant drop in airborne Pb concentrations. In the early 1980's, 5% of Europe's urban population was exposed to air Pb concentrations above the World Health Organization's (WHO) recommended limit of 0.5 $\mu g/m^3$ for an annual average (Fenger, 1999; WHO, 2000). By the late 1980s, this value had fallen, and very few locations reported concentrations above 0.5 $\mu g/m^3$. These areas were primarily near large, uncontrolled metal industries (Fenger, 1999). Notable decreases in airborne Pb have even been seen in remote locations. For example, measurements made in Bermuda between 1993 and 1994 showed that, despite its remote location, airborne-Pb concentrations had fallen by an order of magnitude since the 1970s and by a factor of four since the 1980s (Huang, 1996). Similarly, measurements taken at the South Pole were routinely below the detection limit in 2000-2001, which indicates a significant improvement in Antarctic air quality since the 1970s (Arimoto et al., 2004).

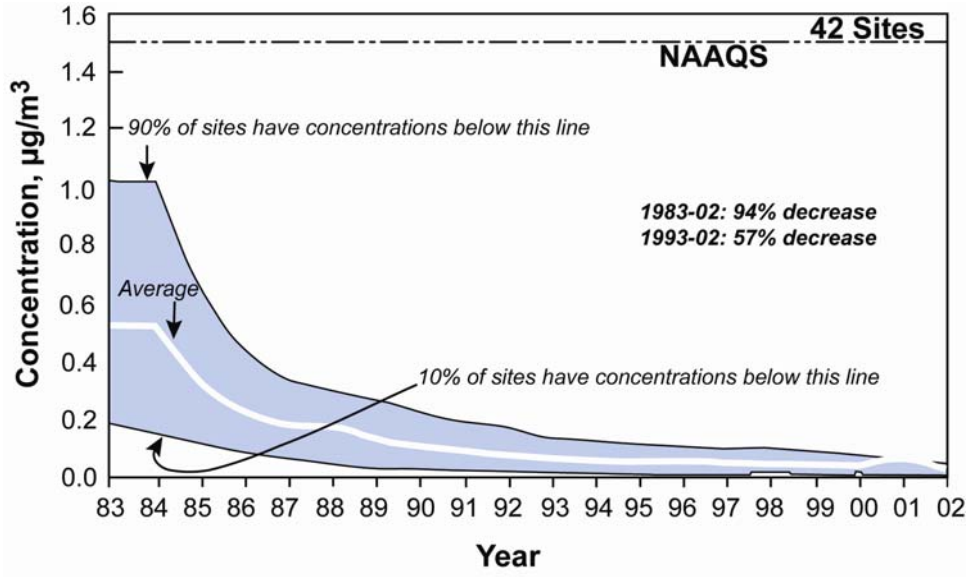


Figure 3-2. Airborne Pb concentrations measured at FRM sites, averaged across the United States for the years 1983 through 2002. The data are plotted in terms of maximum arithmetic mean averaged over a calendar quarter and are shown in relation to the current NAAQS of 1.5 µg/m^3 (quarterly average).

Source: http://www.epa.gov/airtrends/lead.html.

3.1.1 Routine Monitoring of Lead in U.S. Ambient Air

Ambient air Pb concentrations are measured by four monitoring networks in the United States, all funded in whole or in part by EPA. For compliance with the current Pb NAAQS, quarterly average airborne concentrations of Pb are not to exceed 1.5 µg/m^3. Between September 2001 and September 2002, there were just four areas in the United States not in attainment of this standard: Liberty-Acadia, MO; Herculaneum, MO; East Helena, MT; and Lame Deer, MT (U.S. Environmental Protection Agency, 2003). In 2004, there were only two areas out of attainment (www.epa.gov/air/oaqps/greenbk/inte.html).

NAAQS Compliance Monitoring Sites - Federal Reference Method

This network is comprised of official state/local Pb monitoring stations which measure Pb in total suspended particulate matter (TSP), i.e., particles up to about 30 microns, for the purpose of determining compliance with the Pb NAAQS. These stations use samplers and laboratory

analysis methods which have either Federal Reference Method (FRM) or Federal Equivalence Method (FEM) status. The FRM and FEM method descriptions can be found in 40 CFR part 50, Appendix G. Sampling is conducted for 24-hour periods, with a typical sampling schedule of 1 in 6 days. About 250 sampling sites operated during 2005. These sites provide a total Pb measurement and are intended to be used for determining compliance with the Pb NAAQS. The locations of these sites are shown in Figure 3-3. The state/local agencies which operate these sites report the data to EPA's Air Quality System where they are accessible via several web-based tools. Many of the stations in this network have been in operation since the 1970s. EPA's series of annual air quality trends reports have used data from this network to quantify trends in ambient air Pb concentrations. The most recent Trends Report for Lead can be found at http://www.epa.gov/airtrends/lead.html.

Figure 3-3. United States Lead TSP monitoring sites from 2000-2006. Monitor information and data available at: http://www.epa.gov/air/data.

Max quarterly average concentrations of Pb measured at the FRM monitors in 2000 to 2004 are, on average, quite low, with the composite mean ranging from 0.03 to 0.05 μg/m^3 (excluding point source-related monitors) and 0.10 to 0.22 (including point source-related monitors). When data from point source-oriented monitors are included, in any given year during 2000 to 2004 only one to five U.S. locations (from among ~200 sites) had measured max quarterly average Pb levels that exceeded the NAAQS level (1.5 μg/m^3, max quarterly average).

$PM_{2.5}$ Speciation Trends Network

This is a U.S. network of about 200 $PM_{2.5}$ speciation sites. This network consists of 54 long-term trends sites, commonly referred to as the Speciation Trends Network (STN), and about 150 supplemental sites. Nearly all of these state/local sites are in urban areas, often at the location of highest known $PM_{2.5}$ concentrations. Sites in this network determine the Pb concentrations in $PM_{2.5}$ samples and, as such, do not measure Pb in the size fraction >2.5 μm in diameter. Lead is quantified via the XRF method. The standard operating procedure for metals by XRF is available at: http://www.epa.gov/ttnamti1/files/ambient/pm25/spec/xrfsop.pdf. Data are managed through the Air Quality System. These sites generally began operation around 2000.

The locations of these sites are shown in Figure 3-4a; and Figure 3-4b shows the average maximum quarterly mean concentrations of Pb observed at those sites that were at or above 0.005 μg/m^3 for 2002-2005. In these data, the highest max quarterly average Pb concentration reported was 0.168 μg/m^3, and the composite average of max quarterly average concentrations was 0.0080 μg/m^3. As is shown in Figure 3-4b, the max quarterly average Pb concentration exceeded 0.1 μg/m^3 at only one location during this 5-year period.

IMPROVE Network – $PM_{2.5}$ Speciation

In the Interagency Monitoring of Protected Visual Environments (IMPROVE) network, $PM_{2.5}$ monitors are placed in "Class 1" areas (including National Parks and wilderness areas) and are mostly in rural locations. This network is administered by the National Park Service, largely with funding by EPA, on behalf of state air agencies that use the data to track trends in rural visibility. Lead in the $PM_{2.5}$ is again quantified via the XRF method. Data are managed and made accessible mainly through the IMPROVE website, but also are available via the Air

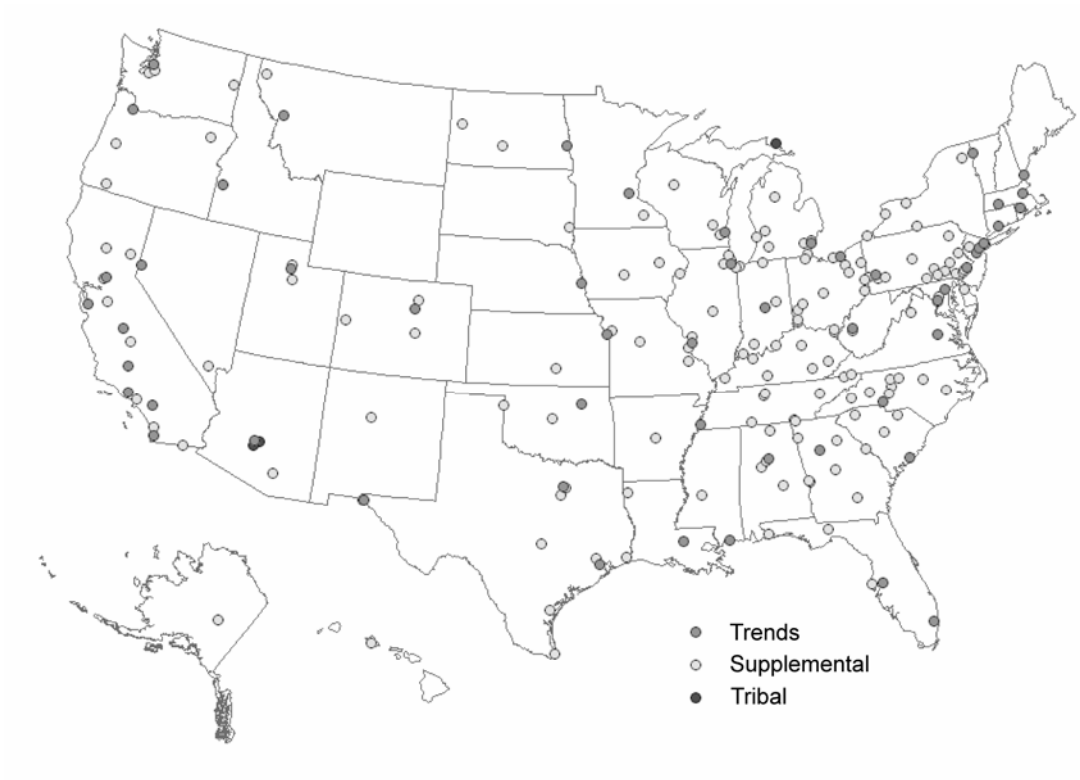


Figure 3-4a. Locations monitored by the Speciation Trends Network (STN).

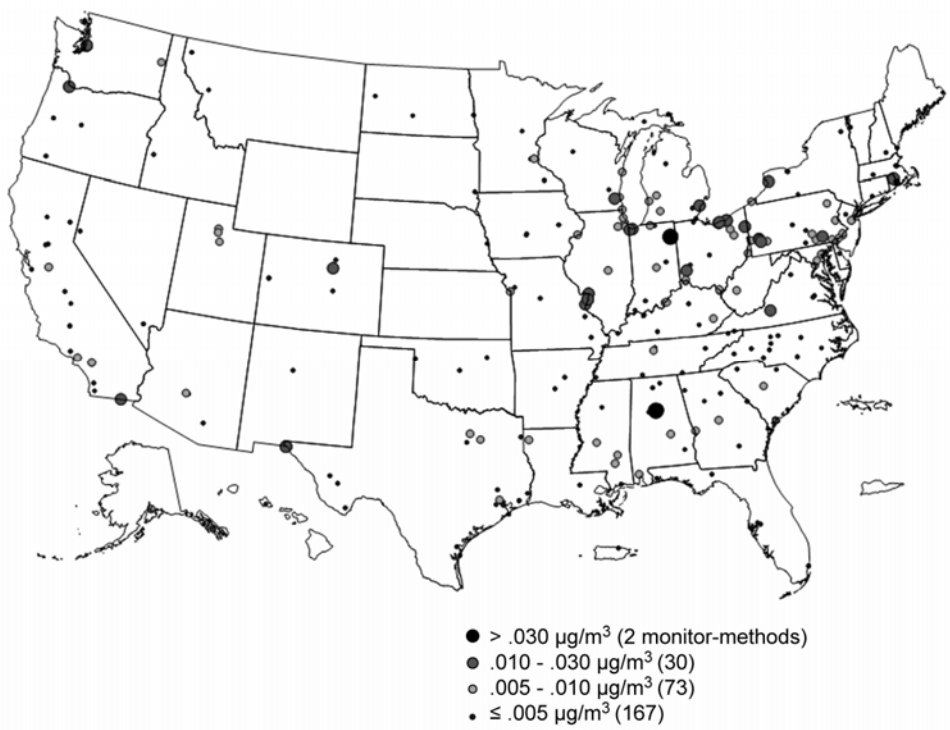


Figure 3-4b. The average maximum quarterly mean Pb concentrations observed in $PM_{2.5}$ by the STN.

Quality System. The oldest of these sites began operation in 1988, while many others began in the mid 1990s. The locations of these sites are shown in Figure 3-5a. There are 110 formally designated "IMPROVE" sites located in or near national parks and other Class I visibility areas, virtually all of these being rural. Approximately 80 additional sites at various urban and rural locations, requested and funded by various parties, are also informally treated as part of the network. Samplers are operated on the same 1 in 3 day schedule as the STN by several different federal, state, and tribal host agencies (see: http://vista.cira.colostate.edu/IMPROVE/).

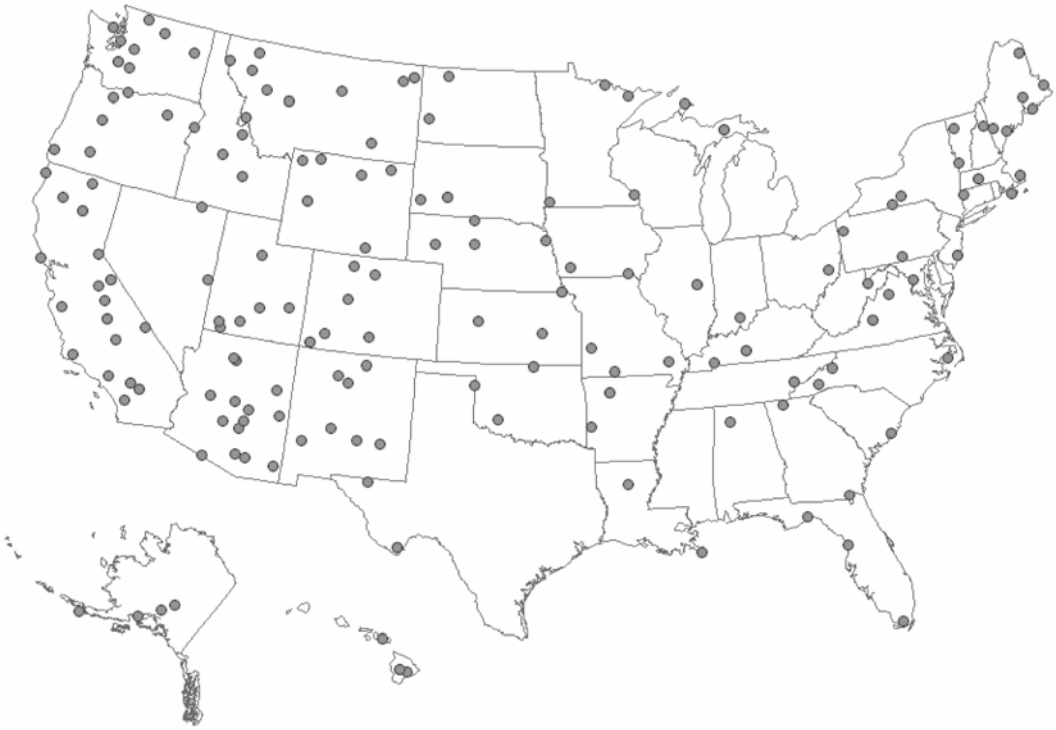


Figure 3-5a. The Interagency Monitoring of Protected Visual Environments (IMPROVE) network of $PM_{2.5}$ monitors. Monitor site information available at: http://vista.cira.colostate.edu/IMPROVE/overview/IMPROVEProgram_files.htm.

Figure 3-5b shows IMPROVE sites that detected ambient air Pb concentrations in $PM_{2.5}$ at or above 0.0008 µg/m^3 between 2000 and 2005. In the data from the IMPROVE network, the highest max quarterly average Pb concentration reported was 0.008 µg/m^3, and the composite

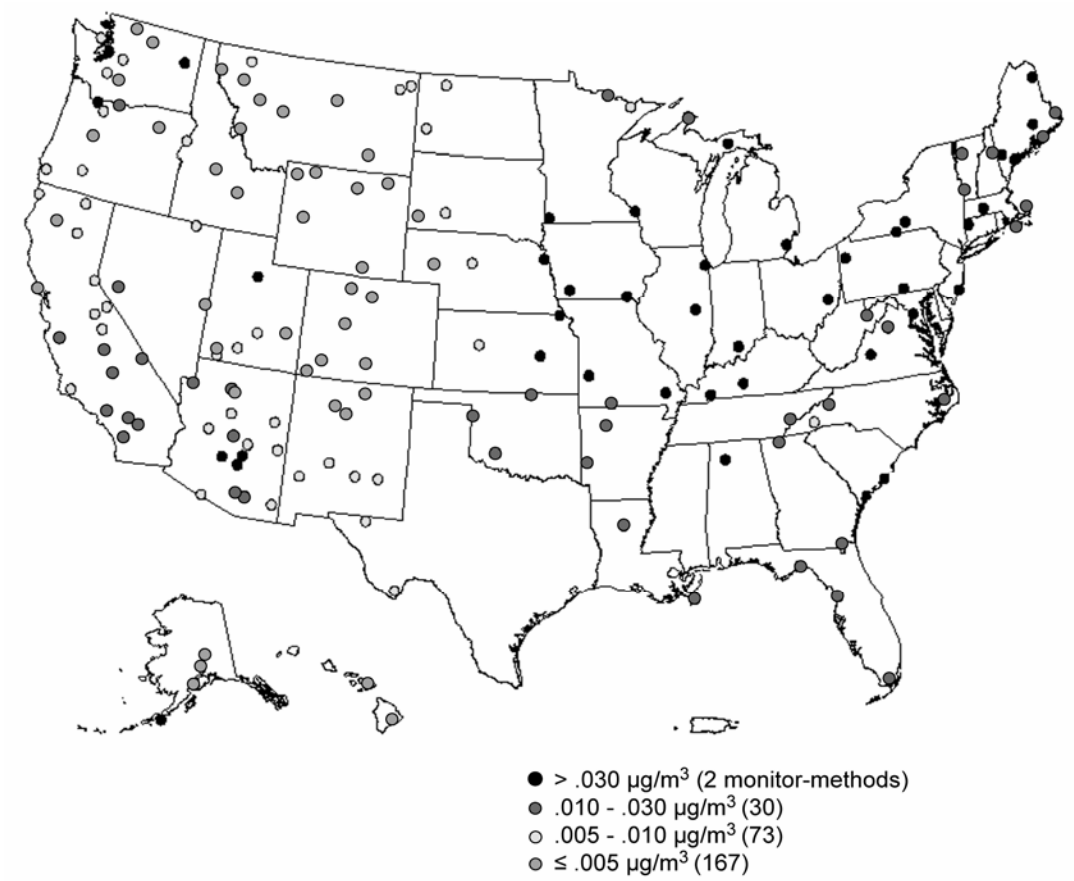


Figure 3-5b. IMPROVE sites with Pb $PM_{2.5}$ concentrations at or above 0.0008 µg/m^3 between 2000 and 2005. Air quality data available at: http://vista.cira.colostate.edu/views/web/General/Data.aspx.

average of max quarterly average concentrations was 0.002 µg/m^3. These levels are considerably lower than those obtained in the $PM_{2.5}$ speciation monitoring network, reflecting the fact that speciation monitors are generally located in urban areas while the IMPROVE sites are in national parks and wilderness areas. Recent studies have also reported that concentrations of airborne Pb are sometimes several orders of magnitude higher in urban areas compared to remote regions (Schroeder et al., 1987; Malm and Sisler, 2000). Rural areas tend to have Pb concentrations falling somewhere between those of urban and remote areas. Thus, urban populations are typically exposed to distinctly higher levels of airborne Pb than rural or remote residents.

National Air Toxics Trends Stations – PM_{10} speciation

The National Air Toxics Trends Stations (NATTS) network of 24 sites monitors mostly urban, but some rural, areas. These sites are also operated by 22 state or local host agencies. All collect particulate matter as PM_{10} for toxic metals analysis and, as such, do not measure Pb in the size fraction >10 µm in diameter. Lead in the collected sample is quantified via the ICP/MS method. The standard operating procedure for metals by ICP/MS is available at: http://www.epa.gov/ttn/amtic/airtox.html. These NATTS sites are relatively new, with 2004 being the first year in which all were operating. The Air Quality System can be accessed at http://www.epa.gov/ttn/airs/airsaqs/ (see Figure 3-6a for the locations of the NATTS monitoring sites).

Figure 3-6a. The National Air Toxics Trends Stations (NATTS) network. Monitor site information available at: http://www.epa.gov/ttn/amtic/airtoxpg.html.

Figure 3-6b shows the arithmetic mean of the maximum quarterly average Pb concentrations in PM_{10} observed at the NATTS sites during 2002 through 2006. In the data from PM_{10} monitors in the NATTS network, the highest max quarterly average Pb concentration observed was 0.039 µg/m^3 during 2002 to 2005, and the composite mean of the max

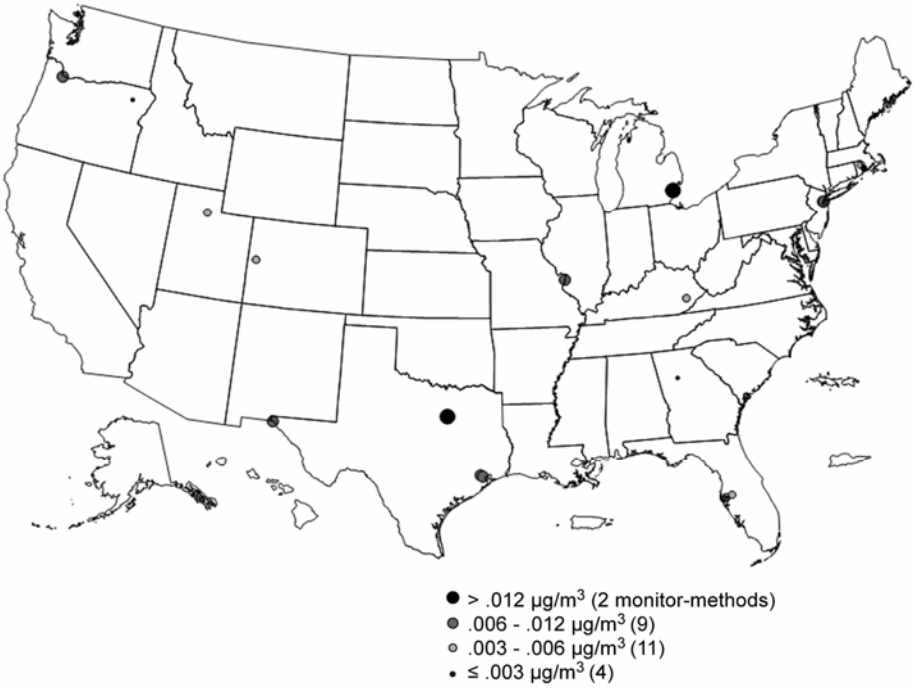


Figure 3-6b. Arithmetic mean of maximum quarterly average Pb concentrations measured in PM_{10} at NATTS network sites during 2002 through 2005. Air quality data available at: http://www.epa.gov/ttn/amtic/airtoxpg.html.

quarterly average concentrations was 0.012 µg/m^3. Data are managed through the Air Quality System.

In addition to these four networks, various organizations have operated other sampling sites yielding data on ambient air concentrations of Pb, often for limited periods and/or for primary purposes other than quantification of Pb itself. Most of these data are accessible via the Air Quality System. In an effort to gather as much air toxics data (including Pb) into one database, the EPA and STAPPA/ALAPCO created the Air Toxics Data Archive. The Air Toxics Data Archive can be accessed at: http://vista.cira.colostate.edu/atda/.

Pb Concentrations in Different PM Size Classes

Airborne Pb concentrations are measured in three PM size fractions, as discussed above – TSP, PM_{10} and $PM_{2.5}$ – by the various monitoring networks. There are not many sites where Pb

measurements are made in different PM size fractions at the same location (and where Pb values exceed the minimum detection limit). From among U.S. monitoring sites, two obtain Pb concentrations in both TSP and PM_{10}, 16 have Pb data available from both TSP and $PM_{2.5}$, and 13 for both PM_{10} and $PM_{2.5}$.

In a combined analysis of data from all collocated monitoring sites, there is typically a good correlation between Pb measurements in TSP and PM_{10} (r = 0.73 at the one site with 10+ paired observations) and between Pb measurements in PM_{10} and $PM_{2.5}$ (r = 0.69 for 11 sites with 10+ paired observations). However, the correlation between Pb measurements in TSP and $PM_{2.5}$ is generally not as high (r = 0.46 for 13 sites with 10+ paired observations). There is substantial variability in the correlation between Pb concentrations in TSP and $PM_{2.5}$ samples at different sites. For those sites with at least 10 paired observations, the correlation coefficients range from −0.25 to +0.95.

Table 3-1 summarizes data for Pb concentrations determined from several particle size fractions in the monitoring networks discussed above. Focusing on the Pb concentrations reported from TSP, PM_{10} and $PM_{2.5}$ samples in urban areas (i.e., not from the IMPROVE network), it can be seen that the mean and median values are not markedly different, though in general $PM_{2.5}$ mass is about 50% of the mass of PM_{10}, which is then about 50% of the mass of TSP depending on the given area.

Table 3-1. Descriptive Statistics for Lead Measurements (in µg/m^3) from Monitors* Using Different Size Fractions of PM for Recent Years**

Particle size (network)	Minimum	Mean	Median	Maximum
TSP*** (FRM, n~200)	0.00	0.01-0.22	0.02-0.04	1.92-9.13
TSP*** (FRM, n~200) excluding point source sites	0.00	0.03-0.05	0.01-0.02	0.26-1.75
PM_{10} (NATTS, n=26)	0.0027	0.0116	0.0101	0.039
$PM_{2.5}$ (Speciation, n=272)	0.002	0.008	0.005	0.168
$PM_{2.5}$ (IMPROVE, n=167)	0.0005	0.0016	0.0013	0.0065

* Excluding monitors representative of point source emissions
** 2000-2004 for data from IMPROVE and TSP; 2002-2005 for data from the $PM_{2.5}$ speciation network and NATTS.
*** Data for TSP presented as range of values for each year

Several research studies have reported Pb concentrations in different PM size fractions. For example, in a rural area in the Southeastern United States, Goforth and Christoforou (2006) reported average Pb concentrations of 6.11 ng/m^3 in $PM_{2.5}$ and 15.04 ng/m^3 in TSP samples. The average total mass of $PM_{2.5}$ and TSP were, respectively, 9.5 µg/m^3 and 19.1 µg/m^3; thus, Pb constituted a similar very small proportion of particles in each size fraction. In another study, Singh et al. (2002) reported concentrations of metals in several PM size classes from two areas in the Los Angeles Basin. In Downey, a site where refineries and traffic contribute heavily to particle concentrations, Pb was proportionally greater in the fine and ultrafine fractions of PM_{10}. In Riverside, which is considered a receptor site for particles transported from the Los Angeles basin and also has agricultural sources, Pb was proportionally greater in the coarse fraction of PM_{10}. In Boston, MA, Pb concentrations of 326 ng/m^3 and 75.6 ng/m^3 were reported from $PM_{2.5}$ and $PM_{10-2.5}$, respectively (Thurston and Spengler, 1985). While there is clearly variation between sites, these findings generally suggest that Pb is somewhat more likely to be found in fine fraction particles than in larger particle sizes.

Lead is measured in three PM size fractions in only a few locations in the United States. Table 3-2 shows Pb concentrations from three such areas: Wayne Co., MI (Detroit); Multnomah Co., OR (Portland); and St. Louis City, MO.

Table 3-2. Maximum Quarterly Mean and Overall Average Quarterly Mean Lead Measurements (in µg/m^3) from U.S. Monitors using Different Size Fractions of PM for Recent Years

Location	Pb in TSP		Pb in PM_{10}		Pb in $PM_{2.5}$	
	Maximum	Average	Maximum	Average	Maximum	Average
Wayne Co., MI	0.041	0.0313	0.039	0.0283	0.0182	0.0165
Multnomah Co., OR	0.015	0.0123	0.0273	0.0188	0.0178	0.0118
St. Louis City, MO	0.216	0.0216	0.0153	0.0143	0.0136	0.0136

Note that although Pb was measured in TSP, PM_{10}, and $PM_{2.5}$ at the same site in each of the above three locations, different PM monitoring methods were used for the different PM size fractions at a given size, contributing to apparent anomaly of PM_{10} Pb value being higher than TSP Pb value for the Oregon site.

Temporal and Spatial Variation in Pb Concentrations

Some seasonal variability is common for air Pb concentrations. However, the extent to which seasonal variability is present depends on precipitation trends, changes in wind direction, and mixing height variability for a given area. For example, a relative maximum was observed in the winter in the Arctic because of the lack of precipitation during winter months (Heidam, 1986), whereas a relative maximum was observed in the summer in Bermuda when winds come predominantly from Africa and Europe (Huang, 1996). Chiaradia and Cupelin (2000) observed no seasonality in Pb concentrations in Geneva, Switzerland. Lead measurements taken at a number of U.S. and French cities suggest some seasonal variation, based on seasonal differences in mixing height (Del Delumyea and Kalivretenos, 1987).

Measurements made in Riverside, CA show diurnal trends (Singh et al., 2002). Lead concentrations are high in the morning (6 to 10 a.m.) and the late afternoon (4 to 8 p.m.). This is most probably indicative of heavy traffic, despite the use of unleaded gasoline, a depressed atmospheric mixing height in the morning, and advection from Los Angeles traffic. Lead concentrations in Riverside are significantly lower during midday (10 a.m. to 4 p.m.) and night (8 p.m. to 6 a.m.).

Concentrations of Pb are dependent on height. This is particularly true if Pb is emitted at street level from traffic. Measurements performed at roadsides in Hong Kong in 1997 showed much higher Pb concentrations at breathing level than at rooftop level (Chan et al., 2000). Similarly, Pb concentrations measured at four elevations in Berne, Switzerland showed a pronounced decrease with height (Gälli and Nyffeler, 1987). Some leaded gasoline was still used in Hong Kong and Switzerland during these two studies. Also, measurements made in an urban street canyon in Lahti, Finland showed that Pb concentrations declined by a factor of five from street level (1.5m) to rooftop level (25m) (Väkevä et al., 1999).

3.1.2 Observed Concentrations – Indoor Air

Concentrations of Pb can be elevated indoors. Lead in indoor air is directly related to Pb in housedust, which poses both an inhalation and an ingestion risk and is discussed in more detail in Section 3.2. Strong correlations have been observed in a Boston study between indoor air, floor dust, and soil Pb concentrations (Rabinowitz et al., 1985a). In the National Human Exposure Assessment Survey (NHEXAS) study of six Midwestern states, Pb concentrations in

personal air were significantly higher than either indoor or outdoor concentrations of air Pb (Clayton et al., 1999). The predominant sources of indoor air Pb are thought to be outdoor air and degraded Pb-based paint.

Lead concentrations tend to be somewhat elevated in houses of smokers. In a nationwide U.S. study, blood-Pb levels were 38% higher in children who exhibited high cotinine levels, which reflect high secondhand smoke exposure (Mannino et al., 2003). Lead is present both in tobacco and in tobacco smoke, although Pb concentrations in tobacco have fallen in parallel with decreases in airborne Pb concentrations (Mannino et al., 2003).

Another source of Pb in residential air is metal-cored candlewicks. The U.S. Consumer Product Safety Commission banned the use of metal-cored candlewicks that contain more than 0.06% Pb as of October 15, 2003 (USGS, 2003). However, prior to this time, Pb emissions from metal-core wicks were measured in the range of 0.5 to 66 µg/hour according to one study (Nriagu and Kim, 2000) and 100 to 1700 µg/hour according to another study (Wassan et al., 2002). In homes where such candles were burned, airborne Pb concentrations could have been well above ambient air levels.

3.1.3 Observed Concentrations – Occupational

Lead concentrations inside work places can also be elevated. Thus, inhalation of Pb during work hours is an additional route of exposure for some subpopulations.

Feng and Barratt (1994) measured Pb concentrations in two office buildings in the United Kingdom (UK). In general, concentrations in the UK office buildings were higher than those in nearby houses. Office dust Pb was concentrated in the organic and residual fractions, unlike house dust which was bound to carbonate and Fe-Mn oxides. This indicates that offices and houses may have different Pb sources. Office building Pb also tends to be in the coarse mode, unlike house dust Pb that predominantly occurs in fine particles (Feng and Barratt, 1994).

As expected, Pb concentrations tend to be highly elevated within manufacturing facilities for Pb-based products (Rieuwerts et al., 1999; Harrison et al., 1981; Tsai et al., 1997). Thus, occupational exposure can represent a major Pb exposure route for employees working in such facilities. For example, measurements taken in a battery manufacturing plant in the Czech Republic found Pb concentrations in floor dust to be 47,700 ppm outside of the assembly plant, 39,200 ppm inside the assembly plant, and 73,700 ppm in the battery grid storage area

(Rieuwerts et al., 1999). In another study in Taiwan, airborne Pb concentrations in a battery manufacturing plant, a metallic film capacitor plant, and a Pb powder plant were 140 ± 112 μg/m^3, 281 ± 114 μg/m^3, and 485 ± 245 μg/m^3, respectively (Tsai et al., 1997). Work sites that use mechanical actions such as abrasion, friction, and cutting typically generate large particles. However, work sites that use high temperature operations generate small, respirable particles. At the three sites listed above, particle sizes were predominantly >10 μm in diameter (Tsai et al., 1997). A Pb-Zn smelter in the UK similarly showed much larger Pb particle sizes inside the facility than outside of the facility (Harrison et al., 1981). This may be because concentrations are high enough indoors to coagulate. Floor dusts (<60 μm) taken from each process site in the overall smelting process contained the same Pb species as the aerosols emitted from each process, which are discussed in Section 2.2.

Residential renovation and paint removal can also be major sources of Pb exposure for both workers and residents. Dry sanding, abrasive blasting, and burning, welding, or heating surfaces covered with Pb-based paint typically generate highly dangerous airborne Pb levels (Jacobs, 1998). Geometric mean and maximum air Pb concentrations observed during each of these processes (as reported by Jacobs, 1998) are listed in Table 3-3. Daniels et al. (2001) measured airborne Pb concentrations during exterior paint removal from residences via wet abrasive blasting technology. The eight-hour, time-weighted average (TWA) air exposures measured via personal monitors ranged between 55.1 and 81.5 μg/m^3. Area air Pb concentrations were between 20.5 and 26.9 μg/m^3.

Lead-based paints were the predominant coating for U.S. highway bridges for many years. Paint removal during bridge renovation projects has also been cited as a major source of Pb exposure for workers. As with residential renovation, Pb concentrations during industrial paint removal depend largely on the technology used. Generally, abrasive blasting techniques are used, which breaks Pb coatings into small particles that can be inhaled or ingested if hands are not washed prior to eating or smoking (Chute and Mostaghim, 1991). Vacuum blasting may reduce occupational exposures. Personal monitors worn during vacuum blasting on a bridge registered air Pb concentrations between 27 and 76 μg/m^3, with a geometric mean of 55 μg/m^3 (Mickelson and Johnston, 1995). Concentrations measured eleven meters from the removal processes fell to 0.1 and 2 μg/m^3 over an eight-hour TWA.

Table 3-3. Airborne Lead Concentrations in Areas Surrounding Residential Lead-Based Paint Abatement Activities

Abatement Technique	Geometric Mean ($\mu g/m^3$)	Maximum Exposure ($\mu g/m^3$)
Preparation (e.g., carpet removal)	2	206
Abrasion	8	403
Chemical stripping	3	476
Encapsulation	2	72
Heat gun	7	915
Component replacement	3	121
Cleaning	2	590

Source: Jacobs (1998).

Certain types of mining operations can also result in occupational Pb exposure. For example, air-Pb concentrations measured in underground gold mines were somewhat elevated, but comparable, to ambient Pb concentrations due to adequate air exchange (Annegarn et al., 1988). Air Pb concentrations ranged between 1.4 $\mu g/m^3$ and 800 $\mu g/m^3$ and were highly dependent on the process being undertaken (Annegarn et al., 1988). However, another source apportionment study in a Nevada gold mine measured Pb concentrations that averaged 0.21 $\mu g/m^3$ (McDonald et al., 2003).

Children of Pb workers are also at increased risk for exposure. In a meta-analysis of take-home Pb exposure, the geometric mean blood Pb level for children was 9.3 µg/dL (Roscoe et al., 1999). This was significantly higher than the geometric mean of 3.6 µg/dL for children overall. Similarly, 52% of children of Pb workers had blood Pb levels at or above 10 µg/dL, compared with just 8.9% of children nationwide (Roscoe et al., 1999). Having a parent in an automobile body or maintenance occupation also appears to contribute to increased blood Pb levels in children (Murgueytio et al., 1998a).

3.2 EXPOSURE: SOIL AND DUST

Contaminated soil can be a potential source of Pb exposure for humans. Soil Pb can be directly ingested through hand-to-mouth behavior common in children, indirectly ingested through contaminated food, or inhaled when breathing air containing resuspended soil particles. Soil ingestion, as reported by parents, peaks during the second year of life and diminishes thereafter (Lanphear et al., 2002). Soil Pb concentrations measured in urban, residential, and industrial areas are discussed here. Soil-Pb concentrations in areas not influenced by Pb sources have been estimated to be in the range of 1 to 200 ppm, with an average of 15 ppm (Zimdahl and Skogerboe, 1977). In a U.S survey, Pb concentrations in agricultural soils were found to range from <1 to 135 mg/kg, with a mean of 12.3 mg/kg (Holmgren et al., 1993). In a review of studies on soil Pb contamination, Markus and McBratney (2001) reported a broad range of soil-Pb concentrations. It should be noted that soil-Pb measurements are difficult to compare, given the variety of extraction techniques and depths of soil cores analyzed in each study.

Lead in soil is derived mainly from atmospheric deposition, both from local sources and long-range transport (Erel et al., 1997; Markus and McBratney, 2001; Sheets et al., 2001). In general, soil in urban and residential areas is contaminated primarily via atmospheric deposition, direct application of agricultural chemicals, and natural mineral weathering of parent rock (Pačes, 1998). At a local level, soil Pb contamination can be derived from agricultural and food wastes, animal wastes and manure, logging and other wood-cutting activities, urban refuse, municipal sewage sludge, miscellaneous organic wastes (including excreta, solid wastes from metal manufacturing, coal fly ash and bottom fly ash, peat for agricultural and fuel uses), wastage of commercial products, mine tailings, and smelter slags and wastes (Nriagu and Pacyna, 1988). Flaking and peeling of Pb-based paint can also be a significant source of soil Pb near old structures (Small et al., 1995; Finkelstein et al., 2003).

The retention time for Pb in the soil is much longer than it is in the air, such that the time in which a change in emissions is reflected in soil Pb concentrations is much longer than for air Pb concentrations. The only "removal" mechanisms for soil Pb are resuspension, mechanical mixing from tilling, landscaping and animals, and leaching, the last of which is known to be a slow process (see Chapter 2 of this document for details). Retention of Pb in soil is influenced by soil characteristics, including the organic matter content (Schwab et al., 2005; Vega et al., 2006). Modeling efforts by Harris and Davidson (2005) in southern California predict that

reduction of surface soil Pb concentration to the steady state concentrations associated with current Pb emissions will not be achieved for more than a hundred years, assuming emission rates stay constant. Miller and Friedland (1994), employing a dynamic analysis, estimated a time to steady state for Pb concentrations in the organic soil horizon of two forest types in the northeastern United States based on estimates of 1989 atmospheric deposition. These times to steady state were estimated to be approximately equal to five response times (5 times the estimate of soil flushing time) or 90 years or 400 years for a northern hardwood forest and a subalpine spruce-fir forest, respectively. The mean response times for Pb in these soils were estimated as 17 and 77 years, respectively. A later study in the same region estimated the response times as 60 years and 150 years for the two forests, respectively (Kaste et al., 2003).

3.2.1 Concentrations of Soil Lead in Urban Areas

The concentration of soil Pb varies significantly throughout urban areas, depending on proximity to stationary sources and roadways and on wind speed and direction. In urban and industrial areas, mean soil-Pb concentrations have been found to range from 23 to 1275 mg/kg, with peak concentrations of over 10,000 mg/kg (Markus and McBratney, 2001).

The major sources of Pb in urban soils are (a) automotive traffic from the days of leaded gasoline (Sheets et al., 2001; Mielke, 1993; Sutherland, 2000) and (b) deteriorating exterior Pb-based paint. Soil concentrations decrease both with depth and distance from roadways. In one study of 831 homes in the United States, 7% of housing units were found to have soil-Pb levels exceeding 1200 ppm, the U.S.EPA/HUD standard for soil Pb concentration outside of play areas (Jacobs et al., 2002). Soil-Pb concentrations were related to the presence of deteriorating exterior Pb-based paint; 24% of housing units that had deteriorated, exterior, Pb-based paint had bare soil-Pb concentrations in excess of 1200 ppm, whereas just 4% of homes without deteriorating paint had soil-Pb levels greater than 1200 ppm (Jacobs et al., 2002). Soil-Pb concentration was also generally higher in homes constructed in earlier years (e.g., before 1940) than more recently constructed homes. Another review of studies conducted in several urban areas concluded that automotive Pb was a more important influence than the presence of Pb-based paint under some conditions (Mielke, 1993).

Extensive studies in Baltimore, New Orleans, and cities throughout Minnesota found the highest soil-Pb concentrations in the central sections of each city, where traffic and population

density are greatest (Mielke, 1991, 1993). The lowest concentrations were found in the outskirts of these cities and in smaller cities. In all of these studies, the age of housing did not seem to be a major factor, which suggests that the impacts of Pb-based paint may be dominated by historic emissions of leaded gasoline additives. However, the fact that the highest concentrations are typically found in the inner city (generally disproportionately populated by minorities and the poor) suggests that these groups are likely most at risk for Pb exposure from contaminated soil.

Some of the highest soil-Pb concentrations are found near major roadways. Surface soil-Pb concentrations measured near a major freeway in Cincinnati, OH, fell between 59 and 1980 ppm (Turer et al., 2001). These concentrations dropped off dramatically with depth. An estimated 40% of Pb from automobile exhaust is retained in the nearby soil (Turer et al., 2001).

Measurements by Erel et al. (1997) in Israel show that soil-Pb concentrations decrease more rapidly with depth near roadways than far from roadways. In a soil profile extracted near a local road, Pb concentrations fell by a factor of 42 between the surface and 30 to 36 cm from the surface. However, far from the roadway, Pb concentrations decreased by about a factor of 3 between the surface and 30 to 36 cm below the surface.

Several authors making measurements during the days of leaded gasoline usage reported elevated Pb concentrations in soil that decreased with distance from roadways. For example, Pierson and Brachaczek (1976) reported soil-Pb levels that decreased from >1000 ppm adjacent to the road down to less than 200 ppm at 12.5 m from the roadway edge. These concentrations have likely stayed high despite the elimination of leaded gasoline use. Harris and Davidson (2005) have also shown through use of a mass balance model that elevated Pb concentrations in soil are likely to remain high for hundreds of years; and this is consistent with other studies showing similarly long residence times for Pb in soil (e.g., Dudka and Adriano, 1997).

Several studies have assessed the impact of soil Pb concentrations on blood-Pb levels. Without accounting for other sources of Pb intake, Duggan and Inskip (1985) estimated that, for every 1000 ppm increase in soil-Pb concentration, children's blood-Pb levels increase 5 µg/dL. Aschengrau et al. (1994) reported decreases in blood-Pb levels of 1.12 to 1.35 µg/dL per 1000 ppm reductions in soil Pb concentrations during a randomized control trial. The results of a pooled analysis of 12 studies showed an average 3.8 µg/dL increase in blood-Pb levels per 1000 ppm increase in soil-Pb levels (Lanphear et al., 1998). Soil abatement at a Superfund site

resulted in a mean 3.5 µg/dL decrease in blood-Pb levels for 6 to 36 month old children (Lanphear et al., 2003). A smaller reduction in blood-Pb levels was observed for 36 to 72 month old children because of age differences, Pb intake from other sources, and mouthing behaviors. Murgueytio et al. (1998b) observed a 2.8 µg/dL increase in blood-Pb levels with increases in soil concentrations of 1000 ppm. Accounting for age differences and, therefore, the redistribution of bone-Pb stores, (Gwiazda et al., 2005) reconciles many of the apparent differences between the results of the Lanphear et al. (1998, 2003), Murgueytio et al. (1998b), and Aschengrau et al. (1994) studies.

3.2.2 Soil-Lead Concentrations Near Stationary Sources

Concentrations Near Lead Smelters

Lead in soil is highly elevated near sources of Pb emissions. In particular, areas around stationary facilities, such as smelters and battery disposal sites, can have very high levels of soil Pb.

Major smelter deposits exist primarily within a 0.5 km radius of the stack (Chatterjee and Banerjee, 1999; Rieuwerts et al., 1999), although some studies have found elevated soil-Pb concentrations as far away as 30 km (Liu, 2003). Franssens et al. (2004) used isotopic measurements to show that between 50% and 80% of dry depositing Pb within a 3 to 4 km radius of a Pb-zinc smelter had an industrial origin.

Soil-Pb concentrations decrease dramatically with distance from the source, and they depend greatly on windspeed and direction (Kimbrough and Suffet, 1995; Palacios et al., 2002; Suchara and Sucharová, 2004). Godin et al. (1985) measured soil-Pb concentrations that were almost proportional to the inverse of the distance from the source and the square root of the wind direction frequency. Suchara and Sucharová (2004) estimated an exponential decrease in soil-Pb concentration with distance from a Pb smelter in the Czech Republic. Data collected within a 14 km radius showed an exponential decrease in soil-Pb concentration with distance from the source. Exponential decreases in soil-Pb concentrations have been suggested elsewhere, as well (e.g., Chatterjee and Banerjee, 1999; Rieuwerts et al., 1999). Results of Chatterjee and Banerjee (1999) indicate that Pb concentrations remain relatively constant within about 250 meters of the source and decrease with distance after this. Examples of data showing decreases in soil-Pb concentration with distance from major sources are shown in Table 3-4.

Table 3-4. Concentrations of Soil Lead with Distance from Lead Smelters

Distance from Smelter (m)	Concentration (ppm, dry weight)
Fenceline	2300[a,4], 46700 ± 2100[a,5], 12650[b,6]
20	5657[d,1]
30	3937[d,1]
40	3253[d,1]
100	783[d,1], 312.8 ± 98.7[e,2], 1800[a,4]
123 - 256	636 ± 522[c,8]
250	229[d,1] , 20200 ± 1100[a,5]
400	127[d,1]
500	400 ± 20[a,5]
700	792[e,7]
1500	519[c,3]
3000	242[c,3]
5000	216.7 ± 87.6[e,2], 137[c,3]
10000	110.3 ± 76.4[e,2]
20000	57.4 ± 24.9[e,2]
30000	32.9 ± 21.4[e,2]

Note: In cases where multiple transects were sampled, only the downwind transects are shown. Values are given as mean ± standard deviation.

[a]Depth sampled was not defined
[b]Sample depth was 0-5 cm
[c]Sample depth was 0-10 cm
[d]Sample depth was 0-15 cm
[e]Sample depth was 0-30 cm

[1]Palacios et al. (2002)
[2]Liu (2003)
[3]Godin et al. (1985)
[4]Kimbrough and Suffet (1995)
[5]Chatterjee and Banerjee (1999)
[6]Rieuwerts et al. (1999)
[7]Venditti et al. (2000)
[8]Young et al. (2002)

As in the case for urban soils, Pb concentrations decrease significantly with depth near industrial sites. As an example, Table 3-5 lists a Pb concentration profile measured near a Pb smelter in northern France.

Table 3-5. Soil Lead Concentration Profile Measured Near a Lead Smelter in Northern France

Depth (cm)	Soil Horizon	Soil Conc. (ppm)
0-6	Oi	2340
6-9	Oa	4480
9-36	Ag	383
36-50	ABg	21.7
50-70	BAg	18.2
70-85	Bg	17.1
85-120	IIC2g	12.4
120-165	IIC3g	10.2

Source: Denaix et al. (2001).

The species of metals found near smelters vary depending on soil conditions. One study observed Pb in topsoil that was either in the form of $Pb_5(PO_4)_3Cl$ or Pb(II) compounds that were adsorbed onto Fe(II) oxides or associated with clay particles (Batonneau et al., 2004). Other measurements at a site contaminated with automotive battery wastes showed Pb species in the soil to be $Pb(CO)_3$, $Pb(CO_3)_2$, $Pb(OH)_2$, PbO, and $PbSO_4$ (Pichtel et al., 2000). Other studies have shown Pb contamination bonded to bacteria (Denaix et al., 2001), carbonate (Maskall and Thornton, 1998; Pichtel et al., 2000; Venditti et al., 2000), sulfide phases (Pichtel et al., 2000; Venditti et al., 2000), organic phases (Pichtel et al., 2000; Venditti et al., 2000) and Fe-Mn oxides (Venditti et al., 2000). The prevalence of carbonate forms in Pb-contaminated soil is due to coinciding contamination with calcareous slag wastes (Maskall and Thornton, 1998).

Soil-Pb concentrations do not appear to have noticeably decreased in areas surrounding smelters despite the implementation of pollution controls. A smelter in Slovenia was fitted with protective filters in 1978 (Zadnik, 2004). Since that time, Pb concentrations have fallen dramatically in hay samples and cow blood within 10 km of the smelter; however, soil-Pb concentrations in areas around the smelter did not decrease between 1978 and 2003 (Zadnik, 2004). Similarly, a Pb-zinc smelter in British Columbia, Canada was replaced by a new smelting facility in 1997 (Hilts, 2003). Airborne Pb concentrations fell by nearly 75%, and Pb levels fell by 50% in outdoor dustfall, street dust, and indoor dustfall fell by 50%. However, no statistically

significant decline was observed in soil-Pb concentrations nor in Pb concentrations in carpeting inside nearby residences (Hilts, 2003). Soil-Pb concentrations at five U.S. factory sites, which had closed decades ago, were elevated as well (Rabinowitz, 2005). Also, many sites where smelters had previously operated but are currently unrecognized as such (Eckel et al., 2001) may represent a previously unidentified Pb exposure risk for nearby populations.

Concentrations Near Mines

Concentrations of Pb are highly elevated near mines as well. Lead and zinc mines, in particular, have large deposits of Pb in nearby soil, but mines used for extracting other metals can also have Pb-contaminated soil nearby. Mine sites are contaminated by the disposal of mine tailings, acid mine drainage, and atmospheric deposition of airborne emissions (Dudka and Adriano, 1997). Mines in the Midwestern United States produced an estimated 480 Tg of Pb tailings and 50 Tg of Pb mine wastes between 1910 and 1981 (Dudka and Adriano, 1997). Concerns about the impact of mine tailing piles from Pb mines are being addressed under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Several Midwestern U.S. states have been allowing the Pb mine waste piles to be used for roadway resurfacing after washing and encapsulation of the waste into asphalt. However, if mine overburden is used for surfacing of unpaved roads, parking lots, highway construction, etc., without first undergoing washing and encapsulation, it can potentially increase the risk for human Pb exposure.

Lead is widely dispersed in areas surrounding mining sites (Dudka and Adriano, 1997; Rieuwerts and Farago, 1995). Thus, it is not easy to determine a relationship between distance and soil concentration, as is the case for smelting emissions. However, a study of an abandoned Pb-zinc mine in Tyndrum, Scotland located near a river showed that fluvial transport had carried Pb contamination at least as far as 6.5 km, although contamination is suspected as far as 25 km downstream (MacKenzie and Pulford, 2002). Examples of soil-Pb concentrations measured near mining sites are shown in Table 3-6.

Lead is found in many different forms near mining sites. It is commonly found in its mineral form of galena (Rieuwerts and Farago, 1995; Dudka and Adriano, 1997). However, in mine spoils, Pb is also found as plumbojarosite [$PbFe_6(SO_4)_4(OH)_{12}$], pyromorphite

Table 3-6. Soil Concentrations Measured Near Mining Sites

Location	Type of Mine	Main Period of Operation	Depth (cm)	Mean conc. (ppm)	Reference
Wales, UK	Pb	historic, not specified	0–15	1159	Gallacher et al. (1984) (taken from Rieuwerts and Farago, 1995)
Halkyn, UK	Pb-Zn	1845–1938	0–15	1127	Davies et al. (1985) (taken from Rieuwerts and Farago, 1995)
Shipham, UK	Zn, Pb	1700–1850	0–15	7900	Mattigod et al. (1986) (taken from Rieuwerts and Farago, 1995)
Shipham, UK	Zn, Pb	1650–1850	0–5	2002	Thornton (1988) (taken from Rieuwerts and Farago, 1995)
Derbys, UK	Pb	18th and 19th cent.	0–5	5610	Thornton et al. (1990) (taken from Rieuwerts and Farago, 1995
Winster, UK	Pb	Up to end of 18th cent.	0–5	7140	Cotter-Howells and Thornton (1991) (taken from Rieuwerts and Farago, 1995)
Leadville, US	Pb	1860s–1960s	n.a.	1110	Cook et al. (1993) (taken from Rieuwerts and Farago, 1995
Derbys, UK	Pb	18th and 19th cent.	0–15	1800	Li and Thornton (1993) (taken from Rieuwerts and Farago, 1995)
Shipham, UK	Zn, Pb	18th and 19th cent.	0–15	7360 (max)	Li and Thornton (1993) (taken from Rieuwerts and Farago, 1995)
Pribram, Czech Republic	Pb	18th–20th cent.	0–5	1451	Rieuwerts and Farago (1996) (taken from Rieuwerts and Farago, 1995)
Tyndrum, Scotland	Pb-Zn	Up to 1862	n.a.	13000	MacKenzie and Pulford (2002)
Goldenville, Canada	Au	1869–1927	n.a.	70–120	Wong et al. (2002)
São Domingos, Portugal	Cu	Pre-Roman–Roman times	0–30	2694	Freitas et al. (2004)
Jasper County, Missouri, U.S.	Pb	1850-1957	n.a.	574 ± 691	Murgueytio et al. (1998b)
Dubuque, Iowa, U.S.	Zn, Pb	19th century	0–20	791	Mbila and Thompson (2004)

[$Pb_5(PO_4)_3Cl$], Pb carbonate [$PbCO_3$], leadhillite [$Pb_4SO_4(CO_3)_2(OH)_2$], $PbS \bullet Bi_2S_3$, Pb oxides, Pb silicates, and Pb sulfate [$PbSO_4$] (Rieuwerts and Farago, 1995; Mbila and Thompson, 2004).

Lead tends to be more heavily concentrated in smaller soil grain sizes than in larger grain sizes (MacKenzie and Pulford, 2002). Results of one study are listed in Table 3-7. Young et al. (2002) observed that the Pb concentration was much higher in the <38 µm size range than in the 300 µm to 2 mm size range in contaminated soils. This is likely due to the higher specific surface area of smaller soil particles and the fact that Pb tends to bond with organic matter and Fe/Al oxides, which can also concentrate in smaller size particles (Young et al., 2002). Also, Rieuwerts and Farago (1995) note that soil-Pb particles are typically larger in mining areas than in smelting areas.

Table 3-7. Concentrations of Lead in Soils Grouped by Soil Grain Size

Size Fraction	Pb conc. of main mine waste	Pb conc. of processing site waste
>180 µm	0.91%	17%
53-180 µm	1.5%	14%
<53 µm	4.5%	18%

Source: MacKenzie and Pulford (2002).

Lead concentrations in peat have also been shown to decrease with depth. Figure 3-7 illustrates two peat profiles sampled near an abandoned Pb mine.

Blood-Pb levels are typically elevated for people living near Pb mines. Soil collected at residences near the Tar Creek Superfund Site, which is a Pb mining area in northeastern Oklahoma, showed wind-dispersed mine wastes (Lynch et al., 2000). More than 20% of soils exceeded the EPA action level of 500 ppm; and children's blood-Pb levels tended to be higher when compared to children living outside the Superfund towns. In this same area, Malcoe et al. (2002) showed that blood-Pb levels were highest among African-American, Mexican-American, and poor children. Blood-Pb levels were most commonly correlated with mean floor dust Pb

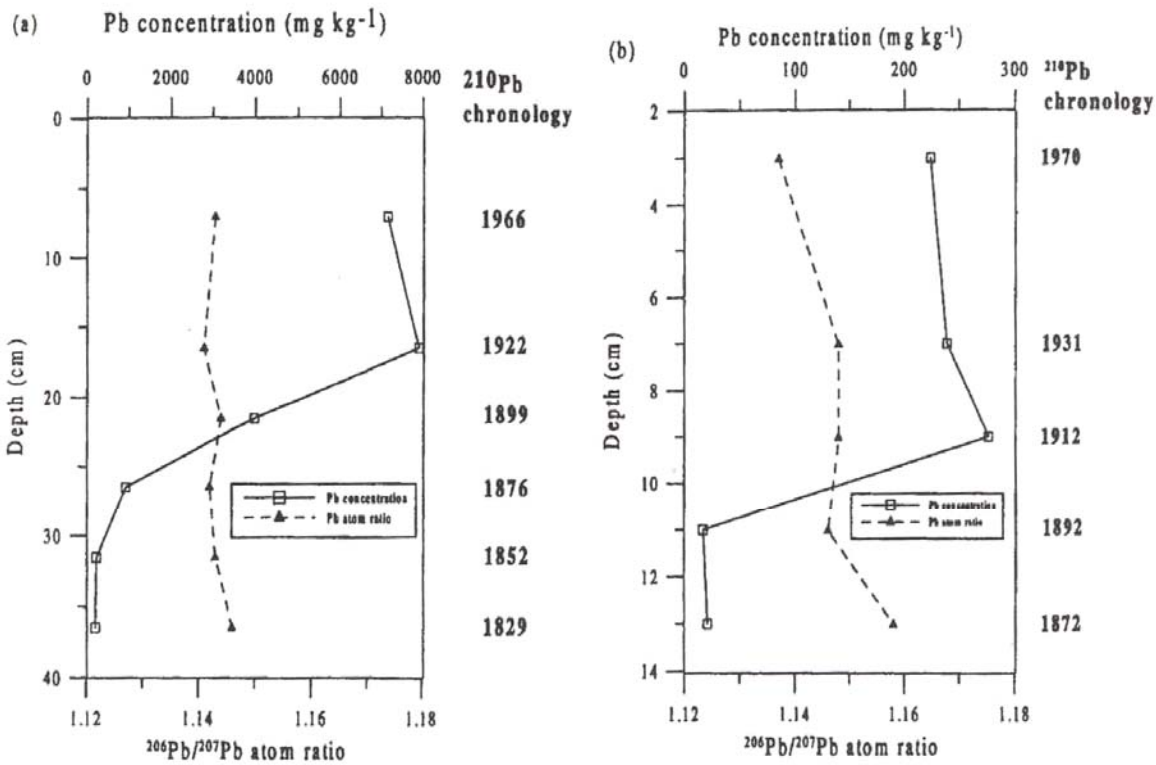


Figure 3-7. The changes in lead concentration with depth in two peat cores. Core A was taken at a location adjacent to the ore processing area of the abandoned lead mine in Tyndrum, Scotland. Core B was taken 0.5 km from the main mine waste dump at the same site.

Source: MacKenzie and Pulford (2002).

loading and with soil Pb, especially front yard soil (Malcoe et al., 2002). At the Jasper County Superfund Site in southwestern Missouri, homes had significantly higher soil and dust Pb levels and significantly higher blood Pb levels than areas outside of the Superfund site (Murgueytio et al., 1998b). A strong statistical relationship observed there between blood-Pb levels and dust-, soil-, and paint- Pb concentrations.

3.2.3 Observed Concentrations – House Dust

Given the large amount of time people spend indoors, exposure to Pb in dusts and indoor air can be significant. For children, dust ingested via hand-to-mouth activity can be a more important source of Pb exposure than inhalation (Adgate et al., 1998; Oliver et al., 1999).

However, dust can be resuspended through household activities (e.g., Ferro et al., 2004), thereby posing an inhalation risk as well. The particle size of "dust" is not well defined, although 50 μm or 75 μm in diameter is sometimes given as an upper limit. In a study performed in the UK, Pb in housedust tended to be bound to the carbonate or Fe-Mn oxides (Feng and Barratt, 1994). Lead can be measured as a concentration in house dust (in μg/g) or as dust Pb loading per surface area (μg/ft^2). One survey reported that dust-Pb loading was a more important predictor of children's blood-Pb concentrations than dust-Pb concentration (Lanphear et al., 1995).

Lead in housedust can derive from a number of different sources. Lead appears both to come from sources outside the home (Jones et al., 2000; Adgate et al., 1998) and from Pb-based paint (Hunt et al., 1993; Lanphear et al., 1996). A chemical mass balance study in Jersey City, NJ observed that crustal sources contributed almost half of the Pb in residences, Pb-based paint contributed about a third, and deposition of airborne Pb contributed the remainder (Adgate et al., 1998). Residential concentrations measured at the Bunker Hill Superfund Site in northern Idaho indicate that the Pb concentration in houses depends primarily on the neighborhood soil-Pb concentration (Von Lindern et al., 2003a, 2003b). However, factors such as household hygiene, the number of adults living in the house, and the number of hours children spend playing outside were also shown to affect Pb concentrations. Using a classification scheme, Hunt et al. (1993) identified sources of Pb in housedust in London for various particle size ranges. In the 64 to 1000 μm size range, the predominant source of Pb was Pb-based paint. However, in the <64 μm size bin, paint, road dust, and garden soil were significant contributors. Lead deposition measured on an interior plate near an open window, an unsheltered exterior plate, and a sheltered exterior plate in New York City were 4.8, 14.2, and 32.3 μg/(ft^2 week) or ~52, 153, and 348 μg/(m^2 week), respectively (Caravanos et al., 2006). Data from a control (interior plate, closed window) showed deposition that was primarily from exterior, environmental sources as well.

Living near a smelter or a mine contributes significantly to the Pb load in residences (Rieuwerts and Farago, 1995; Rieuwerts et al., 1999; Sterling et al., 1998). Homes of mine and smelter employees tend to have Pb levels elevated above those of nearby houses, indicating that Pb can be transported into homes via workers (Rieuwerts et al., 1999). In a U.S., study, mining wastes, paint, and soil were all shown to contribute to housedust Pb (Sterling et al., 1998). Soil

and mining wastes accounted for more than 50% of Pb in housedust. Lead-based paint contributed 16-23% of Pb in housedust in a mining community (Sterling et al., 1998).

Renovation, and especially old paint removal, can greatly increase Pb levels inside the home (Laxen et al., 1987; Jacobs, 1998; Mielke et al., 2001). Removal of exterior paint via power sanding released an estimated 7.4 kg of Pb as dust, causing Pb levels inside one house to be well above safe levels (Mielke et al., 2001). Remaining in a residence during the deleading procedure can be acutely dangerous (Rey-Alvarez and Menke-Hargrave, 1987). Deleading by dry-scraping and sanding has been shown to raise children's blood-Pb levels during the process, but deleading by covering or replacing painted surfaces decreased children's blood-Pb levels during the abatement process (Amitai et al., 1991). Excessive Pb exposure can occur even after Pb abatement. In one prospective controlled study, an average blood-Pb increase of 6.5 μg/dL was observed among children whose homes had undergone Pb-based paint abatement (Aschengrau et al., 1997). Clark et al. (2004) found that despite adherence to U.S. Department of Housing and Urban Development (HUD) post-abatement standards, six-month-old children who lived in houses that had recently undergone Pb abatement were eleven times more likely to have blood-Pb levels increase by 5 μg/dL or more compared to a control group. These studies suggest that existing clearance standards may not be adequate to fully protect children from Pb exposure following abatement or other Pb hazard controls (Clark et al., 2004).

Examples of Pb concentrations and dust Pb loading levels measured in house dust, school dust, and nursing home dust are shown in Table 3-8. It should be noted that dust-Pb loadings may be better predictors of blood-Pb levels than dust concentrations (Lanphear et al., 1995, 1998). Standards for residential Pb loadings of housedust were set by EPA in 2001 to be 40 $\mu g/ft^2$ (430 $\mu g/m^2$) for floors and 250 $\mu g/ft^2$ (2690 $\mu g/m^2$) for windowsills.

An additional concern is attic dust-Pb or dust found in roof cavities. Significant deposits of atmospheric Pb can build up in these spaces. This dust can seep into living spaces through ceiling decorative artwork, cracks between the wall and ceiling, electric light fittings, wall vents, or exhaust, roof, and ceiling fans (Davis and Gulson, 2005). Additionally, renovations, housing additions, ceiling collapses, and storm damage can produce large plumes of attic dust (Davis and Gulson, 2005).

Studies comparing Pb concentrations in attic dust with house age showed a high correlation between attic dust-Pb levels and ambient air-Pb concentration data measured

Table 3-8. Examples of Lead Concentrations and Dust Lead Loadings in Indoor Dust

Concentration or Loading of Lead (ppm unless otherwise indicated)	Location	Surface	Reference
503 (mean)	Edinburgh Scotland	Floor dust	Laxen et al. (1987)
308 (median)	Edinburgh Scotland	Floor dust	Laxen et al. (1987)
43-13,600	Edinburgh Scotland	Floor dust	Laxen et al. (1987)
9 (geometric mean)	Various parts of Denmark	Floor dust	Jensen (1992)
1.5-48.9	Various parts of Denmark	Floor dust	Jensen (1992)
117-362	UK	Floor dust	Feng and Barratt (1994)
1598	Helena and Silver Valleys, US (near 2 Pb smelters)		Schilling and Bain (1988)[a]
3025-4140	Trail, B.C. Canada (near Pb smelter)		Hertzman et al. (1991)[a]
1283	Illinois (near Pb smelter)		Kimbrough et al. (1994)[a]
114-185	Landskrona, Sweden (near Pb smelter)	Floor dust	Farago et al. (1999)[a]
1984	Pribram, Czech Republic (near Pb smelter)	Floor dust	Rieuwerts and Farago (1996)[a]
348	Wales, UK (near a mining site)	Floor dust	Gallacher et al. (1984)[a]
340	Halkyn, UK (near a mining site)	Floor dust	Davies et al. (1985)[a]
786	Shipham, UK (near a mining site)	Floor dust	Thornton (1988)[a]
1870	Derbys, UK (near a mining site)	Floor dust	Thornton et al. (1990)[a]
1560	Winster, UK (near a mining site)	Floor dust	Cotter-Howells and Thornton (1991)[a]
726	Leadville, US (near a mining site)		Cook et al. (1993)[a]
435	Pribram, Czech Republic (near a mining site)	Floor dust	Rieuwerts and Farago (1996)[a]
857 ± 91 ppm in PM_{60}	Jersey City, NJ	Floor dust	Adgate et al. (1998)
1133 ± 119 ppm in PM_{10}	Jersey City, NJ	Floor dust	Adgate et al. (1998)
975 ppm in PM_{53}	Public school in Port Pirie, Australia	Floor dust	Oliver et al. (1999)
481 ppm in PM_{250}	Public school in Port Pirie, Australia	Floor dust	Oliver et al. (1999)

Table 3-8 (cont). Examples of Lead Concentrations and Dust Lead Loadings in Indoor Dust

Concentration or Loading of Lead (ppm unless otherwise indicated)	Location	Surface	Reference
1693-6799 ppm in PM_{53}	Houses in Port Pirie, Australia	Floor dust	Oliver et al. (1999)
1407-4590 ppm in PM_{250}	Houses in Port Pirie, Australia	Floor dust	Oliver et al. (1999)
954	Households in Midwest, US	Windowsill dust	Clayton et al. (1999)
14.4 (ng/m^3)	Households in Midwest, US	Airborne	Clayton et al. (1999)
26.8 (ng/m^3)	Households in Midwest, US	Airborne, personal air	Clayton et al. (1999)
558 ± 544 ppm in TSP	Nursing homes in Vienna	Airborne	Komarnicki (2005)
612 ± 518 ppm in PM_{10}	Nursing homes in Vienna	Airborne	Komarnicki (2005)
547 ± 512 ppm in $PM_{2.5}$	Nursing homes in Vienna	Airborne	Komarnicki (2005)
5140 ($\mu g/m^2$)	Households in Midwest, US	Surface dust	Clayton et al. (1999)
18230 ($\mu g/m^2$)	Households in Midwest, US	Windowsill dust	Clayton et al. (1999)
24,6 ($\mu g/m^2$)	Boston, MA	Floor dust	cited in Lanphear et al. (1998)
3158 ($\mu g/m^2$)	Cincinnati, OH	Floor dust	cited in Lanphear et al. (1998)
219.3 ($\mu g/m^2$)	Cincinnati, OH	Floor dust	cited in Lanphear et al. (1998)
89.3 ($\mu g/m^2$)	Rochester, NY	Floor dust	cited in Lanphear et al. (1998)
191.5 ($\mu g/m^2$)	Rochester, NY	Floor dust	cited in Lanphear et al. (1998)
26.9 ($\mu g/m^2$)	Butte, MT	Floor dust	cited in Lanphear et al. (1998)
20.7 ($\mu g/m^2$)	Bingham Creek, UT	Floor dust	cited in Lanphear et al. (1998)
50.9 ($\mu g/m^2$)	Leadville, CO	Floor dust	cited in Lanphear et al. (1998)
95.5 ($\mu g/m^2$)	Magna, UT	Floor dust	cited in Lanphear et al. (1998)
65.8 ($\mu g/m^2$)	Sandy, UT	Floor dust	cited in Lanphear et al. (1998)
39.6 ($\mu g/m^2$)	Midvale, UT	Floor dust	cited in Lanphear et al. (1998)
63.6 ($\mu g/m^2$)	Palmerton, PA	Floor dust	cited in Lanphear et al. (1998)

[a]Cited in Rieuwerts and Farago (1995).

throughout the lifetime of the house (Chiaradia et al., 1997; Ilacqua et al., 2003). Attic dust may even serve as a proxy for estimating historic ambient Pb concentrations, although the resolution on such calculations would be low. Attic dust-Pb concentrations measured in Australia were an order of magnitude higher in houses near a copper smelter compared to houses far from the smelter (Chiaradia et al., 1997). However, isotopic analyses showed that alkyl-Pb additives were the overall dominant source of Pb contamination in attic dust, suggesting that gasoline emissions had a greater influence than the smelter. The geometric mean concentration of Pb measured in attics in Sydney was 1660 ppm near industrial sites, 1173 ppm near semi-industrial sites, 447 ppm in non-industrial sites, and 16 ppm in background crustal materials (Davis and Gulson, 2005).

Even at low dust-Pb loading levels, Pb in housedust can have an effect on children's blood-Pb levels. Epidemiological studies show that, at a median floor dust-Pb loading level of 5 $\mu g/ft^2$ (54 $\mu g/m^2$), approximately 5% of children have blood-Pb levels $\geq$10 µg/dL (Lanphear et al., 1998, 2005; Malcoe et al., 2002). At a floor dust-Pb loading of 50 $\mu g/ft^2$ (540 $\mu g/m^2$), the percentage of children with blood-Pb levels $\geq$10 µg/dL rose to 20% (Lanphear et al., 1998) In another study, children exposed to floor dust-Pb loadings in excess of 25 $\mu g/ft^2$ (270 $\mu g/m^2$) were at eight times greater risk of having blood-Pb levels $\geq$10 µg/dL compared to children exposed to levels below 2.5 $\mu g/ft^2$ (27 $\mu g/m^2$) (Lanphear et al., 2005).

Throughout early childhood, floor dust-Pb contamination is a source of exposure. Lead-contaminated windowsill dust becomes an additional source of Pb intake during the second year of life when children stand upright. Because of normal mouthing behaviors and increased mobility, the highest blood-Pb levels are seen in children between 18 and 36 months of age (Clark et al., 1991). This peak is typically observed after a rapid rise in blood-Pb levels between 6 and 12 months of age.

3.2.4 Concentrations of Lead in Road Dust

Elevated concentrations of Pb in road dust pose an important exposure risk through wind and traffic resuspension, as outlined in Chapter 2 of this document.

The primary source of Pb in road dust is adjacent soil (De Miguel et al., 1997). However traffic emissions, the weathering and corrosion of building materials (De Miguel et al., 1997), and brake pad wear (Garg et al., 2000) are additional sources. Between 60 to 90% of the mass of

road dust consists of soil particles (Adgate et al., 1998). Soil is still an important reservoir for Pb emitted from vehicles despite the widespread phase out of leaded gasoline. The concentration of Pb in road dust is generally elevated. This is particularly true in urban areas. Additionally, measurements reported in 2003 in the San Joaquin Valley of California show concentrations that were significantly lower than concentrations measured in the same area in 1987 (Chow et al., 2003). Examples of road dust Pb data reported in the literature are listed in Table 3-9. Metals in road dust tend to be associated with small size grains. Measurements of Kuang et al. (2004) show that metals are concentrated in grains smaller than 0.125 mm in diameter.

De Miguel et al. (1997) observed a steep gradient in road dust concentrations of Pb in the north-south direction in Oslo, Norway. This indicates that Pb concentrations are much higher in the highly urbanized areas and lower in the suburban and residential areas. This is consistent with traffic and building construction, renovation, and weathering of building materials being the dominant source of Pb to soil and subsequently road dust (De Miguel et al., 1997).

3.3 EXPOSURE: DRINKING WATER

Lead in drinking water primarily results from corrosion from Pb pipes, Pb-based solder, or brass or bronze fixtures within a residence (Lee et al., 1989; Singley, 1994; Isaac et al., 1997). Very little Pb in drinking water comes from utility supplies. Experiments of Gulson et al. (1994) have confirmed this by using isotopic Pb analysis. Tap water analyses for a public school, apartments, and free standing houses also indicate that the indoor plumbing is a greater source of Pb in drinking water than the utility, even for residences and schools serviced by Pb-pipe water mains (Moir et al., 1996). Ratios of influent Pb concentration to tap concentrations in homes in four municipalities in Massachusetts ranged between 0.17 to 0.69, providing further confirmation that in-home Pb corrosion dominates the trace quantities of Pb in municipal water supplies (Isaac et al., 1997). The information in this section addresses Pb concentrations in water intended for human consumption only. However, such water comes from the natural environment, and concentrations of Pb found in natural systems are discussed in Chapter 7.

The chemical composition of water distribution pipes is of great importance when considering how much Pb is leached into drinking water. Copper piping with Pb-based solder has largely replaced pure Pb piping in the United States. A 1988 survey of 94 U.S. water

Table 3-9. Examples of Observed Road Dust Lead Concentrations

Conc. Of Lead (ppm)	Location	Land Use	Reference
180 ± 14	Oslo, Norway	urban, paved road	De Miguel et al., 1997
1927 ± 508	Madrid, Spain	urban, paved road	De Miguel et al., 1997
536 ± 39	Calcutta, India	near Pb smelter, paved	Chatterjee and Banerjee, 1999
57.2 ± 27.3	Beijing, China	urban, paved road	Kuang et al., 2004
~100	Reno-Sparks, NV	urban, paved road	Gillies et al., 1999
1209 ± 170 ($PM_{2.5}$)	Hong Kong	urban, paved road	Ho et al., 2003
1061 ± 155 (PM_{10})	Hong Kong	urban, paved road	Ho et al., 2003
588 ± 688	Honolulu, HI	urban, paved road	Sutherland et al., 2003
470 ± 524	Honolulu, HI	urban, paved road	Sutherland et al., 2003
151 ± 124	Honolulu, HI	urban, paved road	Sutherland et al., 2003
161 ± 31	San Joaquin Valley, CA	urban, paved road	Chow et al., 2003
57 ± 28	San Joaquin Valley, CA	rural, paved road	Chow et al., 2003
109 ± 74	San Joaquin Valley, CA	composite, paved road	Chow et al., 2003
58 ± 73	San Joaquin Valley, CA	agricultural unpaved road	Chow et al., 2003
203 ± 133	San Joaquin Valley, CA	residential unpaved road	Chow et al., 2003
43 ± 8	San Joaquin Valley, CA	staging area soil	Chow et al., 2003
101 ± 88	San Joaquin Valley, CA	unpaved composite	Chow et al., 2003

companies nationwide revealed that copper pipe was present in 73% of homes, galvanized pipe was present in 13% of homes, a mixture of galvanized and copper was present in 11% of homes, and plastic pipes were present in 2% of homes (Lee et al., 1989). An analysis of PVC pipes indicated that some Pb is leached from PVC in measurable amounts (Sadiq et al., 1997). PVC, which contains ~1% Pb, increased the tap water concentration to an average of 0.017 ± 0.038 mg/L, which was a statistically significant increase over the influent concentration of 0.011 ± 0.026 mg/L (Sadiq et al., 1997). Guo (1997) suggested that Pb may be leached from cement-mortar lined pipes in significant quantities if the cement was made from clinker derived from combusted, hazardous materials.

In addition to piping, Pb may leach from faucets. Water-Pb measurements performed for 12 faucets of different compositions typically found in homes indicated that new cast-brass faucets leached more Pb than any of the other designs (Gardels and Sorg, 1989). Water-Pb levels were below the detection limit from a plastic faucet. In houses with copper piping and Pb-based solder, brass fixtures may contribute as much as 50% of Pb in drinking water (Lee et al., 1989).

The primary type of solder used in the United States was 50–50 tin-Pb solder (50% tin, 50% Pb) before the Safe Drinking Water Act amendments of 1986 were enacted (U.S. EPA 2006). Although new or repaired pipes may not use solder containing more than 0.2% Pb, 50-50 solder still exists in many older structures. In comparing Pb leached from 50–50 tin-Pb solder, 95–5 tin-antimony solder, and a liquefied 50–50 tin-Pb formulation that contained a flux, Birden et al. (1985) showed that the liquefied 50–50 formulation leached the most Pb into drinking water. The 95–5 tin-antimony solder was the safest with respect to drinking water quality. Measurements of metals leached from four, nonlead-based solders in copper pipes were made by Subramanian et al. (1991, 1994). Of the four solders tested (95–5 Sn-Sb, 96-4 Sn-Ag, 94–6 Sn-Ag, and 95.5–4.0-0.5 Sn-Cu-Ag), all showed that metals (Ag, Cd, Cu, Sb, Sn, and Zn) were leached in small enough quantities to make these solders safe alternatives to Pb-based solders.

Lead corrosion is essentially an electrochemical process. Electrons may be transferred from the metal (Pb) to the solution (drinking water), where the major electron acceptors are dissolved oxygen, hydrogen ions, or disinfectant residuals (Singley, 1994). Alternatively, when two different metals are in contact, there is a difference in potential, and the difference in electron demand may increase corrosion (Singley, 1994). In either case, lowering the pH and

increasing the dissolved oxygen demand are known to increase rates of corrosion. The combined pH and alkalinity of water are sometimes described as the aggressiveness of the water and is measured using the Langelier Index. A pH above 8.0 is generally considered safe for Pb leaching (e.g., Lee et al., 1989; Frey, 1989). Also, the corrosion process occurs faster at high temperatures than at low temperatures (e.g., Thompson and Sosnin, 1985; Lee et al., 1989).

There are conflicting reports on the effect of chlorine in water. Chlorine, which is typically used as a disinfectant in municipal supplies, may increase the rate of corrosion by providing a source of electron acceptors (Singley, 1994). However, measurements of Lee et al. (1989) show an absence of statistically significant change in Pb levels with increasing concentration of free chlorine. Laboratory tests of Edwards and Dudi (2004) show that chlorine reacts with soluble Pb^{2+} to precipitate a red-brown colored Pb solid. This solid is highly insoluble, even at a pH of 1.9 for twelve weeks. Thus, chlorine may actually lessen the overall quantity of Pb in drinking water. Elevated levels of Pb in drinking water in Washington DC in 2000 were traced to a change from chlorine to chloramine disinfectant. The red-brown Pb solid does not form in the presence of chloramines, and the data suggest that chloramines dramatically increase the amount of Pb leached from brass (Edwards and Dudi, 2004).

Flouridating water does not seem to affect the solubility or reactivity of Pb compounds (Urbansky and Schock, 2000).

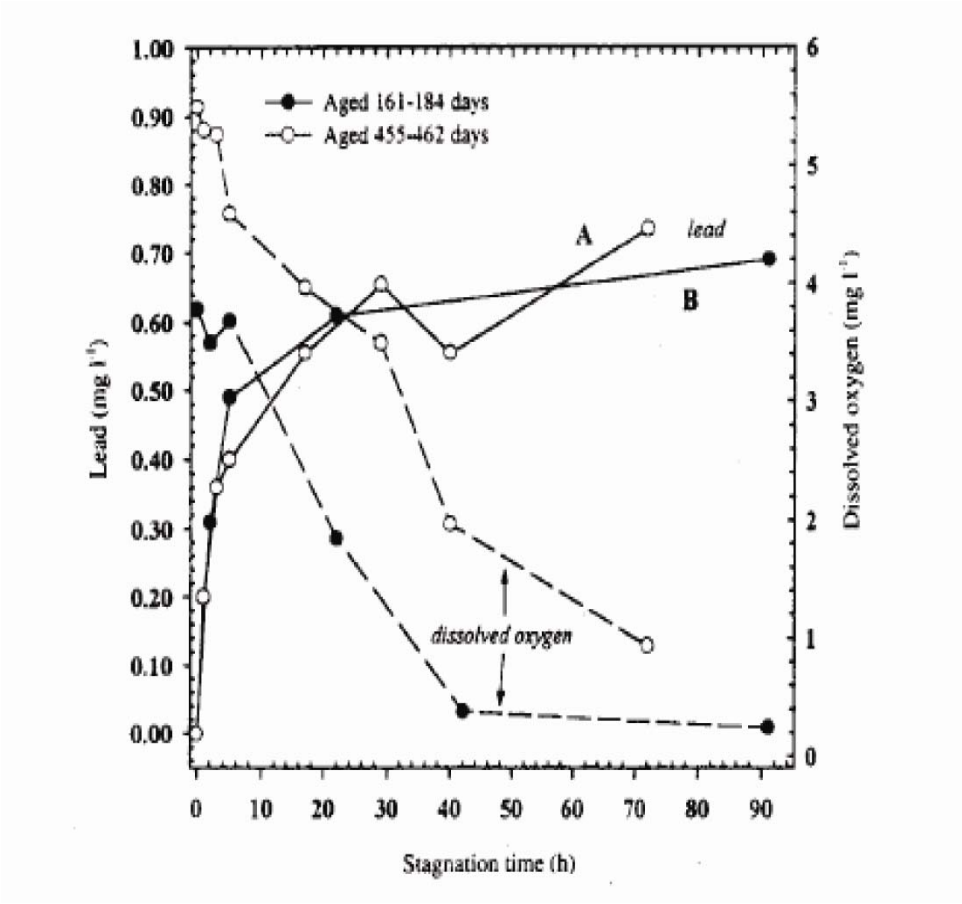
Corrosion inhibitors are sometimes added to water to inhibit scaling or iron precipitation. Zinc orthophosphate in the range of 0.4 to 0.6 mg/L is an effective inhibitor for Pb corrosion (Lee et al., 1989). Results indicate that zinc orthophosphate is more effective at reducing Pb levels than increasing the pH. Soluble Pb release is reduced by up to 70% with the addition of orthophosphate (Edwards and McNeill, 2002). Other proposed corrosion inhibitors such as sodium zinc hexametaphosphate or sodium hexametaphosphate are not effective at reducing Pb corrosion (Lee et al., 1989). In fact, results of McNeill and Edwards (2004) indicate that hexametaphosphate increased the levels of soluble Pb in drinking water. Each milligram per liter of hexametaphosphate increased the Pb content by ~1.6 mg/L after a 72 hour stagnation period in pure Pb pipes (Edwards and McNeill, 2002).

The length of time that drinking water remains in a pipe also affects the water-Pb concentration. Thus, a first flush phenomenon is generally observed in the morning after water has stayed in the pipe through the night. An estimated 47% of total leached Pb was observed in

the first 500 mL of water after prolonged stagnation (Singh and Mavinic, 1991). Gardels and Sorg (1989) demonstrated that 60 to 75% of total Pb leached appeared in the first 125 mL of water after prolonged stagnation. For cold water, the peak Pb concentrations occurred in the first or second 25 mL sample and decreased exponentially with time thereafter. For hot water, the peak Pb concentrations occurred in the second or third 25 mL sample before decreasing exponentially (Gardels and Sorg, 1989). In a system where fully flushed water had a Pb content of 1.7 µg/L, removing just 125 mL of water from the tap every hour kept Pb concentrations elevated (35 to 52 µg/L) throughout the day (Gulson et al., 1997). Lytle and Schock (2000) showed a temporary exponential increase in Pb concentration with stagnation time before the rate leveled off. After 10 hours of stagnation, ~50 to 70% of the maximum Pb concentration had been achieved, although Pb levels continued to increase even after 90 hours of stagnation. Their results are shown in Figures 3-8 and 3-9. It should be noted that the shape of the stagnation-concentration curves was the same for all situations regardless of water quality.

Some examples of Pb concentrations in drinking water are shown in Table 3-10. The Pb standard for drinking water was set by the U.S. EPA in 1988, with a maximum allowable limit of 5 µg/L for water entering the distribution system (Frey, 1989). Longitudinal observations suggest that temporal variation is small for individual households compared to between-home variation (Clayton et al., 1999).

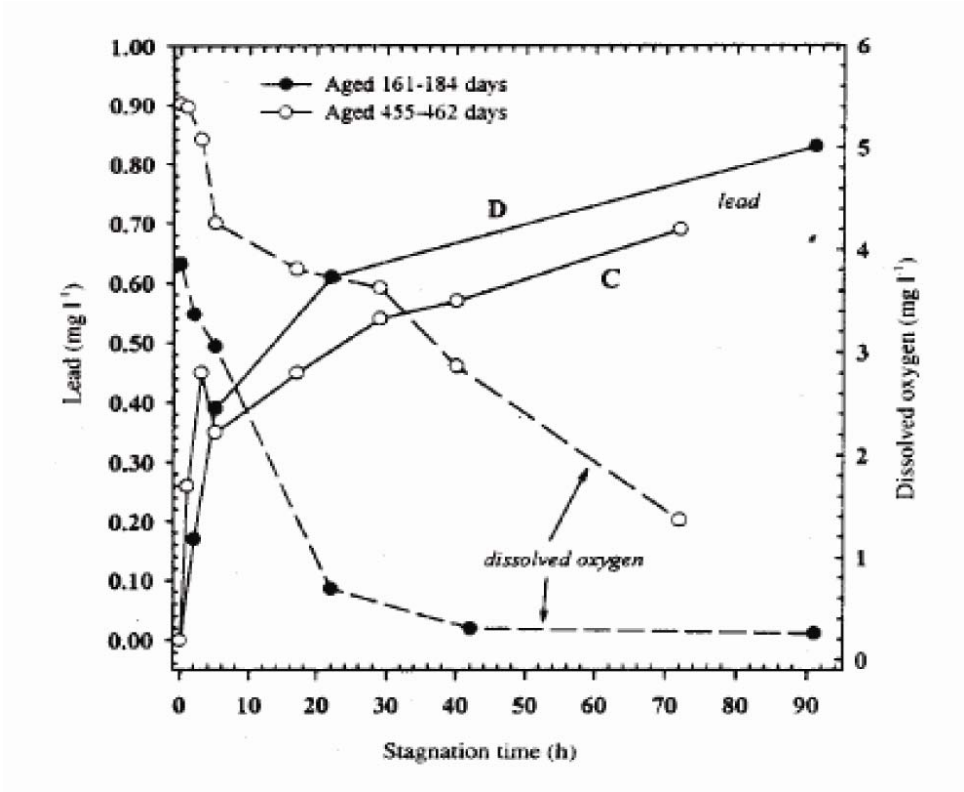
Lead in drinking water can be either in particulate or soluble form. Lead can be in the form of aqueous ions or complexes, particularly when pH is low. Solids are the product of nonadherent corrosion deposits, eroded pieces of plumbing material, or background inputs from the distribution system (Lytle et al., 1993). Lead particles are released when pH and alkalinity are low, and they typically occur in the form of hydrocerrusite scales (McNeill and Edwards, 2004). The main Pb products of corrosion include: CaCO3; PbCO3; Pb3(CO3)2$(OH)_2$; Pb10(CO3)6(OH)6O; Pb5(PO4)3OH; and PbO (Lytle et al., 1993; McNeill and Edwards, 2004). Based on the conditions described above, models to predict drinking water Pb concentrations have been proposed (e.g., Clement et al., 2000; Van Der Leer et al., 2002). Lead in water, although it is typically found at low concentrations in the United States, has been linked to elevated blood-Pb concentrations. In a study of mothers and infants in Glasgow, Scotland tap water was the main correlate of raised maternal blood-Pb levels (Watt et al., 1996). In a prospective study, children exposed to water with Pb concentrations greater than 5 ppb had



Impact of stagnation time on lead and dissolved oxygen concentration in lead pipe (13 mm diameter) exposed to softened water in Study A.

Figure 3-8. The change in lead concentration versus stagnation time. (Reprinted from Lytle and Schock, 2000).

blood-Pb levels ~1.0 µg/dL higher than children with water-Pb levels less than 5 ppb (Lanphear et al., 2002). Thus, water may not be a trivial source of Pb under some conditions. The 1991 EPA Lead and Copper Rule requires that public water utilities conduct monitoring of Pb from customer taps - generally every six months, annually, or triennially, depending on the levels of Pb levels observed in drinking water. Less frequent monitoring is required if levels are low. The rule established a tap water limit ("action level") of 0.015 mg/L (15 ppb) for Pb, based on the 90th percentile concentration, above which corrective action is required (see http://www.epa.gov/safewater/lcrmr/implement.html). The Safe Drinking Water Information



Impact of stagnation time on lead and dissolved oxygen concentration in lead pipe (13 mm diameter) exposed to non-softened water in Study A.

Figure 3-9. Change in lead concentration versus stagnation time. (Reprinted from Lytle and Schock, 2000).

System/Federal Version (SDWIS/FED) maintains a database to which public water utilities are required to submit monitoring data. States have been required to report to EPA the 90th percentile Pb concentrations reported by water systems serving more than 3,300 people. The data available up through 2005 show that about 96% of the utilities that monitored and reported 90th percentile results are below the action level (see http://www.epa.gov/safewater/lcrmr/lead_data.html). For illustrative purposes, Table 3-10 shows 90th percentile drinking water Pb concentrations for a selection of large U.S. cities reported in 1992, 1993, and in more recent years. These are examples of high tap water concentrations that exceed the action level for Pb in tap water and are thusly notably higher than the mean Pb concentrations reported in Table 3-11.

Table 3-10. Examples of Tap Water Concentrations of Lead

Water Concentration (µg/L)	Location	Residence Type	Description	Reference
20	Vancouver, Canada	Apartments	copper or plastic pipes	Singh and Manivic (1991)
13	Vancouver, Canada	Houses	copper or plastic pipes	Singh and Manivic (1991)
0.70	Arizona	Residences	—	Sofuoglu et al. (2003)
0.32	Mexico/US border	Residences	—	Sofuoglu et al. (2003)
16	Halifax, Canada	Houses	standing water	Moir et al. (1996)
8	Halifax, Canada	Houses	running water	Moir et al. (1996)
3	Halifax, Canada	Apartments	standing water	Moir et al. (1996)
2	Halifax, Canada	Apartments	running water	Moir et al. (1996)
6	Halifax, Canada	Public School	standing water	Moir et al. (1996)
5	Halifax, Canada	Public School	running water	Moir et al. (1996)
17	Dharan, Saudi Arabia	Community sites	PVC pipes	Sadiq et al. (1997)
7.7	Clinton, MA	Residences	standing water	Isaac et al. (1997)
25.0	Gardner, MA	Residences	standing water	Isaac et al. (1997)
15.3	Fall River, MA	Residences	standing water	Isaac et al. (1997)
11.6	New Bedford, MA	Residences	standing water	Isaac et al. (1997)
3.92	Midwest, US	Residences	standing water	Clayton et al. (1999); Thomas et al. (1999)
0.84	Midwest, US	Residences	flushed water	Clayton et al. (1999); Thomas et al. (1999)

Table 3-11. 90th Percentile Tap Water Lead Concentrations for a Selection of U.S. Cities Exceeding the EPA Pb Action Level

State	Water system	90th %ile (ppb) 1993	90th %ile (ppb) 1992	90th %ile (ppb) Recent	Recent Monitoring Period
AZ	Phoenix Municipal	19	11	1	1/1/2003 – 12/31/2003
DC	Washington Aqueduct	18	39	63	7/1/2003 – 12/31/2003
FL	Miami Beach, City of	27	4	8	1/1/2001 – 12/31/2001
IA	Cedar Rapids	80	42	6	1/1/2003 – 12/31/2003
IL	Chicago	10	20	7	1/1/1999 – 12/31/2001
MI	Detroit	21	15	12	1/1/2002 – 12/31/2002
MN	Minneapolis	19	32	6	1/1/2002 – 12/31/2002
MN	St. Paul	54	28	11	1/1/2003 – 12/31/2003
NJ	Bayonne Water Dept.	18	25	18	7/1/2001 – 12/31/2001
NY	Syracuse	50	40	25	1/1/2003 – 6/30/2003
NY	Yonkers	68	110	18	1/1/2003 – 6/30/2003
OH	Columbus	15	16	1	1/1/2002 – 12/31/2002
OR	Portland	41	53	8	7/1/2003 – 6/30/2006
PA	Philadelphia Water Dept.	322	15	13	1/1/2002 – 12/31/2002
SC	Columbia, City of	40	114	6	1/1/2002 – 12/31/2002
TX	Galveston	18	6	2	1/1/2000 – 12/31/2002
VA	City of Richmond	16	25	4	1/1/2000 – 12/31/2002
WA	Tacoma	32	17	12	1/1/2001 – 12/31/2003

3.4 EXPOSURE: DIETARY INTAKE

Although notable reductions of Pb in U.S. market basket food supplies have occurred during the past several decades, Pb exposure via consumption of food and beverages can still be a major route of exposure for some groups. As is true for Pb exposure via inhalation, Pb exposure via ingestion has also decreased in the U.S. population. In general, food Pb concentrations have decreased as a direct result of the decrease in airborne emissions of Pb from automotive gasoline, as well as the reduction in the use of Pb solder in cans. In a detailed study of Pb ingestion in food, Flegal et al. (1990) showed that North Americans ingested an estimated

50 µg of Pb each day through food, beverages, and dust; and ~30 to 50% of this amount was from food and beverages. In 1987, the global average daily intake of Pb was estimated to be about 80 µg/day from food and 40 µg/day from drinking water, according to estimates made by the UN Environment Program (Juberg et al., 1997).

More recent data from the Food and Drug Administration's Total Diet Studies show that estimates of daily Pb intake from food dropped substantially between 1982-1984 and 1994-1996 (Egan et al., 2002). In the 1994-1996 Total Diet Study, 74% of samples were found to be below the detection limit for Pb, and daily intake values were presented as ranges reflecting different methods being used to account for measurements below the limit of detection. Infants aged 6-11 months had the lowest estimated dietary Pb intakes; in 1994-1996, intake estimates ranged from 0.8 to 5.7 µg/day (Egan et al., 2002), while in 1982-1984, the average estimated intake was 16.7 µg/day (Gunderson et al., 1988). Similarly, children aged 2 years had estimated dietary Pb intakes in the range of 2.4 to 10.1 µg/day in 1994-1996 (Egan et al., 2002), and Pb intake from the diet was estimated to be larger in 1982-1984, with a mean value of 23.0 µg/day (Gunderson et al., 1988). For older children and adults, dietary Pb intakes in 1994-1996 were in the range of 4 to 19 µg/day (Egan et al., 2002); average dietary intake values in 1982-1984 for adults and children older than 13 years ranged from 28.7 to 41.3 µg/day (Gunderson et al., 1988).

Similar intake levels were observed in a study of children and their mothers in Omaha, Nebraska, where the estimated rate of Pb ingestion was 1.8 µg/day, 3.3 µg/day, 4.1 µg/day and 7.5 µg/day, for age groups of 0 to 12 months, 13 to 24 months, 2 to 6 years, respectively (Manton et al., 2005). In this study, the authors observed that much of the Pb in diet was derived from contamination of foods by household dust (Manton et al., 2005). In one report from the NHEXAS study in Maryland, the mean intake of Pb in the diet was 7.6 µg/day (Scanlon et al., 1999); a subsequent analysis in this study reported a daily dietary intake of 8.14 µg/day (Ryan et al., 2001). The accompanying longitudinal study showed that Pb dietary exposures vary little over time (Scanlon et al., 1999). In the Midwest, Pb concentrations in foods consumed by children 0 to 6 years old were similar or lower than adults, but on a body weight basis Pb intake rates were 1.5 to 2.5 times higher for young children (0.26 µg/kg body weight/day for children 0 to 7 yrs and 0.10 µg/kg body weight for people overall) (Thomas et al., 1999). Overall, a small percentage of the population exceeded health-based intake levels set by FAO/WHO (Thomas et al., 1999). In Australia, women between 20 and 39 years of age ingest between 7.3 and

9.7 µg/day (Gulson et al., 2001a). Infants that are breast-fed take in ~0.73 µg/day compared to 1.8 µg/day for formula-fed infants (Gulson et al., 2001a). Australian children ingest ~6.4 µg/day. Overall, recent studies conducted in the United States indicate that daily Pb intake from the diet ranges from about 1 to 10 µg/day. Some researchers have estimated the contribution of Pb from sources other than food in the diet. For example, Melnyk et al. (2000) estimated a daily Pb intake of 8.4 µg/day in children based on a diet survey, but when they estimated exposure due to the handling of food by children (including Pb from the floor and house dust), the daily Pb intake from ingestion was estimated at 29.2 µg/day.

Potential dietary exposure to Pb can be influenced by a range of factors. Nutrition status and fasting can affect absorption rates. In a study of adult men, increased dietary vitamin D was found to decrease Pb concentration in bone, while increased dietary vitamin C intake was associated with decreased Pb concentrations in blood (Cheng et al., 1998). Fasting conditions have been shown to increase Pb absorption dramatically (Rabinowitz et al., 1980).

Some recent exposure studies have evaluated the relative importance of diet to other routes of Pb exposure. In reports from the NHEXAS, Pb concentrations measured in households throughout the Midwest were significantly higher in solid food compared to beverages and tap water (Clayton et al., 1999; Thomas et al., 1999). However, beverages appeared to be the dominant dietary pathway for Pb according to the statistical analysis (Clayton et al., 1999), possibly indicating greater bodily absorption of Pb from liquid sources (Thomas et al., 1999). Dietary intakes of Pb were greater than those calculated for intake from home tap water or inhalation on a µg/day basis (Thomas et al., 1999). The NHEXAS study in Arizona showed that, for adults, ingestion was a more important Pb exposure route than inhalation (O'Rourke et al., 1999). Egeghy et al. (2005) did not find a significant association between blood Pb concentration and Pb measured in any of the environmental media in the NHEXAS in Maryland; however, the authors note that the short time frame of environmental sample collection was likely not long enough to reflect the long half-life of Pb in the body.

It should be noted that concentrations in food can be very low and are frequently below detection limits. In one study in New Jersey, average Pb concentrations measured in various solid foods and beverages were 20 µg/kg and 3.1 µg/kg, respectively (Melnyk et al., 2000). Examples of Pb concentrations measured in several foods are shown in Table 3-12. In the Food and Drug Administration's Total Diet Study, dietary intake of Pb was distributed among grains,

Table 3-12. Examples of Lead Concentrations in Food Products

Food	Concentration	Location	Description	Reference
Barley, grain	0.4 ppm		Uncontaminated soil	Dudka and Miller (1999)
Barley, grain	2.0 ppm		Zn-Pb smelter contaminated	Dudka and Miller (1999)
Potato tubers, peeled	0.21 ppm		Uncontaminated soil	Dudka and Miller (1999)
Potato tubers, peeled	0.89 ppm		Zn-Pb smelter contaminated	Dudka and Miller (1999)
Lettuce	0.19 ppm		Edible portion, untreated soil	Dudka and Miller (1999)
Spinach	0.53 ppm		Edible portion, untreated soil	Dudka and Miller (1999)
Potatoes	0.03 ppm		Edible portion, untreated soil	Dudka and Miller (1999)
Wheat	0.02 ppm		Edible portion, untreated soil	Dudka and Miller (1999)
Rice	0.01 ppm		Edible portion, untreated soil	Dudka and Miller (1999)
Sweet corn	0.01 ppm		Edible portion, untreated soil	Dudka and Miller (1999)
Field corn	0.01 ppm		Edible portion, untreated soil	Dudka and Miller (1999)
Carrots	0.05 ppm		Edible portion, untreated soil	Dudka and Miller (1999)
Onions	0.04 ppm		Edible portion, untreated soil	Dudka and Miller (1999)
Tomatoes	0.03 ppm		Edible portion, untreated soil	Dudka and Miller (1999)
Peanuts	0.01 ppm		Edible portion, untreated soil	Dudka and Miller (1999)
Soybeans	0.04 ppm		Edible portion, untreated soil	Dudka and Miller (1999)
Applesauce, canned	8.5 µg/serving		FDA Total Diet Study	Juberg et al. (1997)
Fruit cocktail, canned	7.1 µg/serving		FDA Total Diet Study	Juberg et al. (1997)
Spinach, fresh	2.4 µg/serving		FDA Total Diet Study	Juberg et al. (1997)
Peaches, canned	6.0 µg/serving		FDA Total Diet Study	Juberg et al. (1997)

Table 3-12 (cont'd). Examples of Lead Concentrations in Food Products

Food	Concentration	Location	Description	Reference
Pears, canned	4.9 µg/serving		FDA Total Diet Study	Juberg et al. (1997)
Strawberries, fresh	1.1 µg/serving		FDA Total Diet Study	Juberg et al. (1997)
Apple juice, bottled	2.6 µg/serving		FDA Total Diet Study	Juberg et al. (1997)
Wine	7.7 µg/serving		FDA Total Diet Study	Juberg et al. (1997)
Vaccinium vitis-idaea	0.4-2.3 ppm	Monchegorsk, Russia	Berry, near Ni-Cu smelter	Barcan et al. (1998)
Vaccinium myrtillus	0.7-1.6 ppm	Monchegorsk, Russia	Berry, near Ni-Cu smelter	Barcan et al. (1998)
Rubus chamaemorus	0.3-4.7 ppm	Monchegorsk, Russia	Berry, near Ni-Cu smelter	Barcan et al. (1998)
Empetrum hermaphroditum	0.3-1.5 ppm	Monchegorsk, Russia	Berry, near Ni-Cu smelter	Barcan et al. (1998)
Leccinum auranticcum	0.8-2.3 ppm	Monchegorsk, Russia	Mushroom, near Ni-Cu smelter	Barcan et al. (1998)
Leccinum sacbrum	1.1-5.2 ppm	Monchegorsk, Russia	Mushroom, near Ni-Cu smelter	Barcan et al. (1998)
Russul vesea	1.1-3.4 ppm	Monchegorsk, Russia	Mushroom, near Ni-Cu smelter	Barcan et al. (1998)
Xerocomus subtomentosus	1.3-3.1 ppm	Monchegorsk, Russia	Mushroom, near Ni-Cu smelter	Barcan et al. (1998)
Suillus luteus	2.0-2.3 ppm	Monchegorsk, Russia	Mushroom, near Ni-Cu smelter	Barcan et al. (1998)
Lactarius trivialis	1.1-3.1 ppm	Monchegorsk, Russia	Mushroom, near Ni-Cu smelter	Barcan et al. (1998)
Lactarius torminosus	0.6-3.5 ppm	Monchegorsk, Russia	Mushroom, near Ni-Cu smelter	Barcan et al. (1998)
Lettuce	0.65-1.3 ppm	Copenhagen, Denmark	Close to lead smelter	Moseholm et al. (1992))
Lettuce	0.15-0.46 ppm	Copenhagen, Denmark	Far from lead smelter	Moseholm et al. (1992))
Lettuce	0.36 ppm	Copenhagen, Denmark	Background concentration	Moseholm et al. (1992))
Carrots	0.07-0.28 ppm	Copenhagen, Denmark	Close to lead smelter	Moseholm et al. (1992))
Carrots	<0.02-0.09 ppm	Copenhagen, Denmark	Far from lead smelter	Moseholm et al. (1992))

Table 3-12 (cont'd). Examples of Lead Concentrations Food Products

Food	Concentration	Location	Description	Reference
Carrots	0.02-0.03 ppm	Copenhagen, Denmark	Background concentration	Moseholm et al. (1992))
Potatoes	<0.02-0.12 ppm	Copenhagen, Denmark	Close to lead smelter	Moseholm et al. (1992))
Potatoes	<0.02-0.06 ppm	Copenhagen, Denmark	Far from lead smelter	Moseholm et al. (1992))
Potatoes	<0.02 ppm	Copenhagen, Denmark	Background concentration	Moseholm et al. (1992))
Kale	1.4-9.3 ppm	Copenhagen, Denmark	Close to lead smelter	Moseholm et al. (1992))
Kale	0.58-2.4 ppm	Copenhagen, Denmark	Far from lead smelter	Moseholm et al. (1992))
Kale	0.52-0.72 ppm	Copenhagen, Denmark	Background concentration	Moseholm et al. (1992))
Wine	65 µg/L	France	Vintage 1990-1995	Médina et al. (2000)
Breast milk	0.55 µg/kg	Australia		Gulson et al. (2001b)
Infant formula	1.6 µg/kg	Australia		Gulson et al. (2001b)
Baby food	2.9 µg/kg	Australia		Gulson et al. (2001b)
Brassica juncea	298.3 ppm	Taihe, China	Indian mustard, near lead smelter	Cui et al. (2003)
Triticum aestivum L.	19.2 ppm	Taihe, China	Common wheat, near lead smelter	Cui et al. (2003)
Basil	<10 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Cabbage	<10 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Cilantro	49 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Collard greens	12 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Coriander	39 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Ipasote	14 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Lemon balm	20 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)

Table 3-12 (cont'd). Examples of Lead Concentrations in Food Products

Food	Concentration	Location	Description	Reference
Mint	<10 - 60 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Mustard greens	<10 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Parsley	<10 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Red chard	<10 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Rhubarb	<10 - 36 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Sage	<10 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Swiss chard	22-24 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Thyme	<10 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Carrot	10 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Onion	21 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Radish	12-18 ppm	Chicago, IL	Edible portion, urban garden	Finster et al. (2004)
Tuna, canned	0.1 ppm (max.)			Lourenço et al. (2004)
Sardines, canned	0.2 ppm (max.)			Lourenço et al. (2004)
Blue mussel, canned	0.3 ppm (max.)			Lourenço et al. (2004)
Balsamic vinegar	15-307 µg/L			Ndung'u et al. (2004)
Wine vinegar	36-50 µg/L			Ndung'u et al. (2004)
Tea leaves	0.59-4.49 ppm	Zhejiang Province, China	Commercial tea producing areas	Jin et al. (2005)
Cocoa beans	0.5 ng/g	Nigeria		Rankin et al. (2005)
Cocoa, manufactured	230 ng/g	Nigeria		Rankin et al. (2005)
Chocolate products	70 ng/g	Nigeria		Rankin et al. (2005)

fruits, mixtures and sweets; dairy foods contributed at least 10% of Pb intake by infants and children to age 16, and beverages contributed between 8 and 18% of Pb intakes by adults (Egan et al., 2002). Ryan et al. (2001) assessed the contribution from various foods to dietary Pb intake, and found that numerous different items, including both canned and fresh fruits and vegetables, cereal and hamburger, were predictive of blood-Pb concentrations. Using the Dietary Exposure Potential Model, Moschandreas et al. (2002) reported that drinking water, coffee and tea (likely reflecting Pb from the drinking water source) were major contributors to estimated daily dietary Pb intake.

One review article concluded that, since the elimination of Pb solder in U.S. canned food, the primary source of Pb in U.S. food is atmospheric deposition (Flegal et al., 1990). Overall, anthropogenic aerosols account for an estimated 40% of Pb in food, while the bulk of the remainder is derived from harvesting, transporting, processing, packaging, or preparing the food (Flegal et al., 1990; Juberg et al., 1997; Dudka and Miller, 1999). Lead contamination in poultry and livestock is also primarily atmospheric in origin. Lead deposits on forage or feed or onto soil that is directly ingested (Flegal et al., 1990). Lead concentrations in food have been reported to increase by a factor of 2 to 12 between harvest and consumption (Flegal et al., 1990). A food production facility in Turkey was shown to contaminate pasta with Pb (Demirözü and Saldamli, 2002), as indicated by Pb concentrations in the semolina being 14.2 to 36.5 ng/g compared with the finished pasta product where concentrations ranged from 107.1 to 147.6 ng/g (Demirözü and Saldamli, 2002). An increase (from an average value $\leq$0.5 ng/g to average values between 11.9 and 69.8 ng/g) between raw and finished cocoa products has also been observed (Rankin et al., 2005). In this case, contamination seems to occur during shipping and/or processing.

Other significant sources of dietary Pb are calcium-supplemented food where calcium is derived from limestone and tin coatings that contain Pb.

Lead concentrations in vegetables may be increased by soil amendments such as mine wastes, slag, or fly ash. Historically, mine tailings were often disposed in streambeds, and this poses an exposure risk when stream sediments are used to boost productivity in gardens (Cobb et al., 2000). Slag is sometimes used for constructing agricultural and forestry roads or for landfill. This can be an additional source of Pb contamination for nearby crops (Bunzl et al., 2001). Fly ash is applied to land infrequently for alkaline adjustment, as cover for landfills, or to amend agricultural soils. Elevated Pb levels in fly ash can subsequently contaminate crops

(Brake et al., 2004). Although soil contamination may be important on a local scale, overall atmospheric deposition is a more significant source of food Pb than uptake from soil. For example, more than 52% of the total Pb present in citrus fruits was removed by washing, indicating that surface deposits make up the bulk of Pb contamination in unprocessed fruit (Caselles, 1998).

3.5 LEAD-BASED PAINT

Lead-based paint was the dominant form of house paint for many decades, and a significant percentage of homes still contain Pb-based paint on some surfaces. As discussed in previous sections, Pb-based paint can be an important source of Pb in house dust and soil, thus contributing to human exposure through those routes. Lead-based paint also poses a potential exposure risk due to inhalation during renovation or demolition projects, or due to ingestion from hand-to-mouth activities and pica, which are common in children. Lead-based paint exposure is one of the most common causes of clinical Pb toxicity.

In a 1970 study, it was observed that for children with blood-Pb levels >50 µg/dL, more than 80% were reported to ingest paint chips or broken plaster (Sachs et al., 1970). A later study by McElvaine et al. (1992) showed that children with blood-Pb levels above 55 µg/dL were ten times more likely to have paint chips observable on abdominal radiographs than children with blood-Pb below this value. Shannon and Greaf (1992) noted that the majority of preschool-aged children with blood-Pb over 25 µg/dL were reported to put paint chips in their mouth.

As Pb-based paint degrades, it becomes incorporated into house dust, which was discussed in depth earlier in this chapter. Lead-based paint can pose an inhalation risk during renovation and demolition activities. As described in Section 3.1 of this document, renovation projects often involve abrasive blasting techniques to remove old layers of paint. This forms Pb particles that are easily inhaled (Chute and Mostaghim, 1991; Mickelson and Johnston, 1995; Jacobs, 1998; Mielke et al., 2001). At industrial sites, exposure is limited primarily to workers. However, during residential renovation or abatement projects, residents may be unduly exposed to very high levels of airborne Pb. Blood-Pb levels were shown to increase in children who lived in houses with a significant (>1.5 mg/cm² on at least one surface) amount of Pb paint that had

undergone some sanding, scraping, or other indoor surface refinishing in the preceding six months (Rabinowitz et al., 1985b).

Additionally, exterior Pb-based paint can degrade and contaminate nearby soil. Paint and surface soil samples collected in and around Oakland, CA households of children having elevated blood-Pb levels had nearly identical isotopic ratios as the children's blood (Yaffe et al., 1983). This suggests that weathered, Pb-based paint was a major exposure route for these children who played outside near the most highly contaminated areas.

3.6 OTHER ROUTES OF EXPOSURE

3.6.1 Calcium Supplements

Potentially toxic levels of Pb were measured in calcium supplements in studies undertaken in the 1960s through the early 1990s (Scelfo and Flegal, 2000). An analysis of 136 different brands of supplements showed that two-thirds of the supplements did not meet the 1999 California criteria for acceptable Pb levels: 1.5 µg Pb/daily dose of calcium (Scelfo and Flegal, 2000). The lowest concentrations were observed in calcium products that were nonchelated synthesized and/or refined. These corresponded to antacids and infant formulas. Antacids and infant formulas had Pb concentrations ranging from below the detection limit to 2.9 µg Pb/g calcium (Scelfo and Flegal, 2000). Natural calcium supplements derived from bonemeal, dolomite, or oyster shell were much more likely to be in exceedance of the 1999 standard. Pb levels reported elsewhere showed comparable Pb levels in supplements and cow milk (Juberg et al., 1997). Whole milk, 2% milk, and calcium supplements had Pb concentrations in the range of 1.7 to 6.7 µg Pb/g calcium, 0.8 to 9.0 µg Pb/g calcium, and 3.1 to 6.9 µg Pb/g calcium, respectively (Juberg et al., 1997). A clamshell powder commonly known as hai gen fan that is added to tea has shown detectable levels of Pb contamination as well (CDC, 1999).

3.6.2 Glazes

Lead glazes have been commonly used throughout history. Kitchen glassware cannot have a Pb solubility in excess of 2.5 to 7 µg/mL according to a 1980 rule by the U.S. Food and Drug Administration (Flegal et al., 1990). However, Pb glazes on imported pottery may persist.

Foods with low pH (acidic ones) are particularly susceptible to solubilizing Pb and thereby contaminating food during storage in Pb glazed glassware. Lead glazes may be especially problematic when low temperature fluxes and glazes are used. This is more common in traditional charcoal kilns than gas-fired kilns.

3.6.3 Miniblinds

Some imported vinyl miniblinds form Pb dust upon disintegration (Juberg et al., 1997). This exposure route was responsible for several cases of Pb poisoning in Arizona and North Carolina in the mid 1990s. Lead stabilizers are not used in vinyl miniblinds manufactured in the United States (Juberg et al., 1997).

3.6.4 Hair Dye

The analysis of Mielke et al. (1997) shows that some hair dyes contain Pb acetate in the range of 2300 to 6000 µg Pb/g of product. This Pb can be easily transferred via hand-to-mouth and hand-to-surface activity, and an estimated 3 to 5% of Pb acetate can be transferred through the skin. Hair dyes tested in this study contain 3 to 10 times more Pb than is allowable for paint (Mielke et al., 1997).

3.6.5 Other Potential Sources of Lead Exposure

Additional consumer products that may pose a risk of Pb exposure include Pb crystal, pool cue chalk (Miller et al., 1996), cosmetics, and folk remedies, which purposefully contain Pb (such as alarcon, alkohl, azarcon, bali goli, coral, gliasard, greta, kohl, KooSo or KooSar pills, liga, pay-loo-ah, rueda, and surma of East Indian, Pakistani, Chinese, and Latin American origins) (CDC, 1999). Unintentional or malicious Pb contamination has also been found for the following products: ground paprika, ayurvedic metal-mineral tonics, and Deshi Dewa (a fertility drug) (CDC, 1999; Kakosky et al., 1996).

3.7 MEASUREMENT METHODS

Methods for measurement of Pb in environmental media were discussed extensively in the 1986 Lead AQCD, and the reader is referred to that document for details regarding the main methods employed and associated detection limits. Some of the most commonly employed methods are concisely noted below.

The concentration of Pb in air can be measured through several different methods. Use of filter media and inertial impactors are two of the most common methods, and in both cases particles can be separated by size. An additional method involves mounting a particle separation device in the stack along with gas flow control and metering equipment. The collected particles are then analyzed for mass and Pb content (Clarke and Bartle, 1998).

Sampling of airborne particles to determine concentration and chemical species can be performed via direct-reading instruments, which include optical counters, electrical counters, resonant oscillation aerosol mass monitors, and beta radiation detectors (Koutrakis and Sioutas, 1996). Additionally, particles may be collected in cyclones and denuder systems.

Collected particles can be analyzed for Pb using x-ray fluorescence analysis (XRF), proton-induced x-ray emission (PIXE), neutron activation analysis (NAA), atomic absorption (AA), or inductively-coupled plasma mass spectrometry (ICP-MS) (Koutrakis and Sioutas, 1996).

Lead concentrations in soil, dust, food, and other environmental media are determined using similar techniques. Generally, substances undergo acid digestion in an HCl or HNO_3 solution before analysis via XRF, PIXE, NAA, AA, or ICP-MS. Special care should be taken in all cases to avoid external contamination of samples, especially when measuring very low Pb concentrations.

For detailed discussions of methods for determining Pb speciation and isotopic ratios, see Chapter 7 of this document. Also, see Chapter 4 for information related to measurement of Pb in biological tissues.

3.8 SUMMARY

Lead concentrations in various environmental media have been discussed throughout this chapter. Concentrations of Pb in all environmental media are present in detectable quantities. In general, Pb exposure in the United States has fallen with the elimination of leaded gasoline, Pb-based paint and Pb solder in cans. However, the potential for high Pb exposures remains, particularly in areas near major Pb sources or with exposures to Pb-based paint or high Pb levels in drinking water.

As airborne concentrations of Pb have fallen in the United States, a corresponding decrease in blood-Pb levels of the U.S. population has been observed. In a meta-analysis of 19 studies from six continents, a strong linear correlation was observed between blood-Pb levels and gasoline Pb levels (Thomas et al., 1999). As gasoline Pb was reduced to zero in the study countries, airborne Pb concentrations declined and converged to less than 0.2 $\mu g/m^3$, and blood-Pb levels also declined, converging to a median of 3 µg/dL.

A U.S. exposure study, the National Human Exposure Assessment Survey (NHEXAS), has included assessment of the relationship between blood-Pb concentrations and exposure measurements in various media as well as activity or household variables. In the NHEXAS in Arizona, total daily Pb intake was found to range from 11 to 107 µg/day, with a mean of 36 µg/day (O'Rourke et al., 1999).

The relative contribution of Pb different media to human exposure varies, particularly for different age groups. In the NHEXAS-Arizona study of a largely adult population, food was the predominant source of Pb exposure, followed by consumption of beverages and tap water; most measurements of Pb in indoor air, outdoor air, soil and dust were below the detection limit (O'Rourke et al., 1999). In a study of children 6 to 24 months of age, Pb in floor dust had the largest association with PbB concentrations, followed by Pb in drinking water and Pb in soil (Lanphear et al., 2002).

The highest air, soil, and road dust concentrations are found near major Pb sources, such as smelters, mines, and heavily trafficked roadways. While airborne Pb concentrations have declined dramatically with the phase out of leaded gasoline, soil concentrations have remained relatively constant, reflecting the generally long retention time of Pb in soil. Soil-Pb concentrations decrease both with depth and distance from roadways and sources such as smelters or mines. In another study of 831 homes in the United States, 7% of housing units were

found to have soil-Pb levels exceeding 1200 ppm, the U.S.EPA/HUD standard for soil-Pb concentration outside of play areas (Jacobs et al., 2002).

Drinking water is susceptible to Pb contamination primarily through leaching from pipes, solder, and faucets. Water that has been stagnant in pipes, has been disinfected with chloramines, has a low pH, or has a low alkalinity is particularly high risk for leaching Pb.

Lead-contaminated food can be an important exposure route. Deposition of airborne Pb and house dust are the major sources of Pb in food. Data from the Food and Drug Administration's Total Diet Studies show that estimates of daily Pb intake from food has dropped substantially in recent years; for example, estimated dietary Pb intake dropped 96 percent in 2- to 5-year old children (from 30 µg/day to 1.3 µg/day) between 1982-1984 and 1994-1996 study periods (Egan et al., 2002). Across all age groups, estimated Pb intake ranged from 0.8 to 19.6 µg/day, with the lowest intake estimates in infants aged 6-11 months (Egan et al., 2002).

Lead-based paint is still prevalent in older homes. This can be a major exposure route if paint has deteriorated or undergone careless renovation. Lead-based paint also can be an important source of Pb exposure through soil or house dust.

Other sources of Pb exposure vary in their prevalence and potential risk. These include calcium supplements, Pb-based glazes, certain types of miniblinds, hair dye, and other consumer products.

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4. TOXICOKINETICS, BIOLOGICAL MARKERS, AND MODELS OF LEAD BURDEN IN HUMANS

4.1 INTRODUCTION

The preceding two chapters presented important background information on the physical and chemical properties of lead (Pb) and its inorganic and organic compounds, sources and emissions of Pb into the ambient air and other environmental media, and concentrations of Pb in ambient air and other components of multimedia human exposure pathways (e.g., water, food, soil, exterior and interior/dusts, etc.). This chapter deals predominately with the relationship between human Pb exposure and Pb burden in the body.

With exposure, mainly by ingestion and inhalation, a portion of Pb is absorbed and distributed to various body compartments from which it is eliminated at various rates. Conceptually, the body's Pb burden may be considered to be divided between a fast (soft tissues) and a slow (skeletal) compartment. Lead in blood exchanges with both of these compartments. The contribution of bone Pb to the blood Pb changes with the duration and intensity of the exposure, age, and various physiological variables (e.g., nutritional status, pregnancy, and menopause).

In Pb toxicologic and epidemiologic studies, dose-response relationships for nearly all of the major health effects of Pb are typically expressed in terms of an index of internal Pb dose. Blood Pb concentration is extensively used in epidemiologic studies as an index of exposure and body burden mainly because of the feasibility of incorporating its measurement into human studies relative to other potential dose indicators, e.g., Pb in kidney, plasma, urine, or bone. Blood Pb is determined by both the recent exposure history of the individual, as well as the long-term exposure history that leads to accumulation in bone. The benefits and limitations of blood Pb concentration as an indicator of Pb body burden were discussed in Section 13.3.2 of the 1986 Lead AQCD. Application of internal dose-response information to the assessment of risks from Pb exposures requires means for estimating the resultant internal doses. Approaches to estimating external Pb exposure impacts on internal tissue concentrations, including various

types of regression analyses and complex biokinetic modeling, are thusly topics of much importance here.

This chapter begins by providing an overview of the toxicokinetics of Pb, focusing on our current understanding of the routes of Pb exposure, uptake, distribution, and elimination in humans. Next, there is a detailed discussion of biological markers used to assess human Pb burden and exposure. Subsequently, models for assessing Pb exposure-burden relationships in humans are presented. The modeling discussion begins with recent developments in epidemiological models of Pb exposure-blood Pb concentration relationships in humans. The evolution of Pb biokinetics modeling and other major modeling advances during the past 25 years or so is then discussed.

4.2 TOXICOKINETICS OF LEAD

Information on Pb toxicokinetics was extensively summarized in the 1986 Lead AQCD (U.S. Environmental Protection Agenyc, 1986) and has been the subject of several recent reviews (e.g., ATSDR, 2005; Mushak, 1991, 1993). Since the completion of the 1986 AQCD, knowledge of the Pb toxicokinetics has advanced in several areas. For example, new studies have been published on the kinetics of Pb movement into and out of bone (based on analysis of stable Pb isotope profiles) that have demonstrated the importance of bone Pb stores as an internal source of Pb to the blood, fetus, and nursing infant. New animal and human experimental models have been developed for studying dermal and gastrointestinal (GI) bioavailability of Pb; the latter studies have provided a more quantitative understanding of the GI bioavailability of Pb in soils. Also, several new models of the toxicokinetics of Pb in humans have been developed that incorporate simulations of bone growth and resorption in the distributional kinetics of Pb in humans.

The summary provided below discusses the major features of Pb absorption, distribution, metabolism, and excretion. Information specific to route of exposure (e.g., inhalation, oral, dermal) is discussed under separate subsections. Distinguishing features of inorganic and organic Pb (e.g., alkyl Pb compounds) are also discussed.

4.2.1 Absorption of Lead

Inhalation Exposure

Inorganic Lead

Inorganic Pb in ambient air consists of aerosols of Pb-bearing particulate matter (PM). Amounts and patterns of deposition of inhaled particulate aerosols in the respiratory tract are affected by the size of the inhaled particles, age-related factors that determine breathing patterns (e.g., relative contributions of nose and mouth breathing), airway geometry, and air-stream velocity within the respiratory tract (James et al., 1994). Publicly available models can be used to predict the deposition and clearance of Pb-bearing particles in the respiratory tract of children and adults. Two such models currently in wide use were developed by (1) the International Commission on Radiological Protection (ICRP, 1994) and (2) the CIIT Centers for Health Research (CIIT), USA, in collaboration with the National Institute of Public Health and the Environment (RIVM), the Netherlands, and with the Ministry of Housing, Spatial Planning and the Environment, the Netherlands. The CIIT model or the Multi-Path Particle Dosimetry (MPPD, available at http://www.ciit.org/mppd) model has been described in detail elsewhere (Anjilvel and Asgharian, 1995; Asgharian et al., 2004; Brown et al., 2005; de Winter-Sorkina and Cassee, 2002). Both the ICRP and the MPPD model can be used to predict deposition for particles of ~0.01 μm to 20 μm in diameter. The reader is referred to ICRP (1994) for detailed information on factors affecting particle deposition and clearance in the human lung as well as on breathing patterns as a function of age and activity. Absorption of Pb deposited in the respiratory tract is influenced by particle size and solubility, as well as by the pattern of regional deposition within the respiratory tract. Fine particles (<1 μm) deposited in the bronchiolar and alveolar region can be absorbed after extracellular dissolution or can be ingested by phagocytic cells and transported from the respiratory tract (Bailey and Roy, 1994). Larger particles (>2.5 μm) that are primarily deposited in the ciliated airways (nasopharyngeal and tracheobronchial regions) can be transferred by mucociliary transport into the esophagus and swallowed.

Quantitative studies of the deposition and clearance of inhaled inorganic Pb in humans have been limited to studies of adults and to exposures to relatively small particles (Chamberlain et al., 1978; Hursh and Mercer, 1970; Hursh et al., 1969; Morrow et al., 1980; Wells et al., 1977). In these studies, exposures were to Pb-bearing particles having mass median

aerodynamic diameters (MMAD) below 1 µm. Deposition of inhaled Pb particles of this size can be assumed to have been primarily in the bronchiolar and alveolar regions of the respiratory tract (James et al., 1994), where mucociliary transport is likely to have been a relatively minor component of particle clearance (Hursh et al., 1969), compared to the fate of larger particles. Approximately 25% of inhaled Pb chloride or Pb hydroxide (MMAD 0.26 and 0.24 µm, respectively) was deposited in the respiratory tract in adult subjects who inhaled an inorganic Pb aerosol through a standard respiratory mouthpiece for 5 min (Morrow et al., 1980), whereas ~95% of deposited inorganic Pb inhaled as submicron particles was absorbed (Hursh et al., 1969; Wells et al., 1977). Clearance from the respiratory tract of inorganic Pb inhaled as submicron particles of Pb oxide or Pb nitrate was multiphasic, with approximate half-times of 0.8 (22%), 2.5 (34%), 9 (33%), and 44 h (12%) (Chamberlain et al., 1978). Given the submicron particle size of the exposure, these rates are thought to represent, primarily, absorption from the bronchiolar and alveolar regions of the respiratory tract. As noted previously, amounts and rates of absorption of inhaled Pb particles that are larger than 2.5 µm, and which may be more typical of certain human exposure scenarios, will be mainly determined by rates of mucociliary transport to the GI tract.

While no quantitative studies of the deposition and absorption of inhaled Pb in children have been reported, the behavior of Pb-containing particles in the respiratory tract can be inferred from experimental studies of inert particle deposition in children and particle dosimetry models. The effect of an individual's age on extrathoracic particle deposition is not well characterized. For example, Bennett et al. (1997) reported ~50% greater deposition of 4.5-µm (MMAD, mass median aerodynamic diameter) particles in children relative to adults. Xu and Yu (1986) also predicted increasing deposition of 2-µm MMAD particles with decreasing age. In contrast, Becquemin et al. (1991) reported nasal deposition to increase with age for particles between 1 and 3 µm MMAD. Both experimental and modeling studies show little difference in thoracic deposition fraction between adults and children at natural resting breathing patterns (Asgharian et al., 2004; Bennett and Zeman, 1998; Hofman et al., 1989; Phalen and Oldham, 2001). Of importance with regard to surface doses is that particle deposition is greater in the tracheobronchial region and lower in the pulmonary region of children compared to adults (Hofman et al., 1989; Phalen and Oldham, 2001). Normalized to lung volume, total deposition is predicted to be greatest in infants and decreases with age for particle between 0.01 and 10 µm

(Asgharian et al., 2004). This increase in deposition per unit lung volume was predominately attributable to particle losses in the tracheobronchial airways. Bennett and Zeman (1998) also noted that because children have smaller lungs and higher minute ventilation relative to lung size, they would be expected to receive greater particle doses per lung surface area compared to adults.

Organic Lead

Alkyl Pb compounds can exist in ambient air as vapors. Inhaled tetraalkyl Pb vapor is nearly completely absorbed following deposition in the respiratory tract. Following a single exposure to vapors of radioactive (^{203}Pb) tetraethyl Pb (~1 mg/m^3 breathed through a mouthpiece for 1 to 2 min) in four male subjects, 37% of inhaled ^{203}Pb was initially deposited in the respiratory tract, of which ~20% was exhaled in the subsequent 48 h (Heard et al., 1979). One hour after the exposure, ~50% of the ^{203}Pb burden was associated with liver, 5% with kidney, and the remaining burden widely distributed throughout the body, suggesting near complete absorption of the Pb that was not exhaled. In a similar experiment conducted with ^{203}Pb tetramethyl Pb, 51% of the inhaled ^{203}Pb dose was initially deposited in the respiratory tract, of which ~40% was exhaled in 48 h. The distribution of ^{203}Pb 1 h after the exposure was similar to that observed following exposure to tetraethyl Pb.

Oral Exposure

Inorganic Lead

The extent and rate of GI absorption of ingested inorganic Pb are influenced by physiological states of the exposed individual (e.g., age, fasting, nutritional calcium and iron status, pregnancy) and physicochemical characteristics of the Pb-bearing material ingested (e.g., particle size, mineralogy, solubility, Pb species). Lead absorption may also vary with the amount of Pb ingested.

Effect of Age. Gastrointestinal absorption of water-soluble Pb appears to be higher in children than in adults. Estimates derived from dietary balance studies conducted in infants and children (ages 2 weeks to 8 years) indicate that ~40 to 50% of ingested Pb is absorbed (Alexander et al., 1974; Ziegler et al., 1978). In adults, estimates of absorption of ingested water-soluble Pb compounds (e.g., Pb chloride, Pb nitrate, Pb acetate) ranged from 3 o 10% in

fed subjects (Heard and Chamberlain 1982; James et al., 1985; Rabinowitz et al., 1980; Watson et al., 1986); absorption fraction in fasted adults is high (~50% increase, see Effect of Fasting). Data available on Pb absorption between childhood and adulthood ages are very limited. While no absorption studies have been conducted on subjects in this age group, the kinetics of the change in stable isotope signatures of blood Pb in mothers and their children as both come into equilibrium with a novel environmental Pb isotope profile, suggests similar ingested Pb absorption fractions in children (ages 6 to 11 years) and their mothers (Gulson et al., 1997). The mechanisms for an apparent age difference in GI absorption of inorganic Pb have not been completely elucidated and may include both physiological and dietary factors (Mushak, 1991).

Studies in experimental animals provide additional evidence for an age-dependency of GI absorption of Pb; however, not all studies have completely segregated possible age-differences in absorption from age differences in elimination of absorbed Pb. Absorption of Pb, administered as Pb acetate (6.37 mg Pb/kg, oral gavage), was higher in juvenile rhesus monkeys (38% of dose) compared to adult female monkeys (26% of the dose) (Pounds et al., 1978). Rat pups absorb ~40 to 50 times more Pb via the diet than do adult rats (Aungst et al., 1981; Forbes and Reina, 1972; Kostial et al., 1978). This age difference in absorption may be due, in part, to the shift from the neonatal to adult diet, and to postnatal physiological development of the intestine (Weis and LaVelle, 1991).

Effect of Fasting. The presence of food in the GI tract decreases absorption of water-soluble Pb (Blake and Mann, 1983; Blake et al., 1983; Heard and Chamberlain, 1982; James et al., 1985; Maddaloni et al., 1998; Rabinowitz et al., 1980). In adults, absorption of a tracer dose of Pb acetate in water was ~63% when ingested by fasted subjects and 3% when ingested with a meal (James et al., 1985). Heard and Chamberlain (1982) reported nearly identical results. The arithmetic mean of reported estimates of absorption in fasted adults was 57% (based on Blake et al., 1983; Heard and Chamberlain, 1982; James et al., 1985; Rabinowitz et al., 1980). Reported fed:fasted ratios for absorption in adults range from 0.04 to 0.2 (Blake et al., 1983; Heard and Chamberlain, 1983; James et al., 1985; Rabinowitz, et al., 1980). Mineral content is one contributing factor to the lower Pb absorption when Pb is ingested with a meal; i.e., the presence of calcium and phosphate in a meal depresses absorption of ingested Pb (Blake and Mann, 1983; Blake et al., 1983; Heard and Chamberlain, 1982).

Effect of Nutrition. Lead absorption in children is affected by nutritional iron status. Children who are iron-deficient have higher blood Pb concentrations than similarly exposed iron-replete children, suggesting that iron deficiency may result in higher Pb absorption or, possibly, other changes in Pb biokinetics that contribute to altered blood Pb concentrations (Mahaffey and Annest, 1986; Marcus and Schwartz, 1987). Evidence for the effect of iron deficiency on Pb absorption has been derived from animal studies. In rats, iron deficiency increases the GI absorption of Pb, possibly by enhancing binding of Pb to iron-binding proteins in the intestine (Bannon et al., 2003; Barton et al., 1978a; Morrison and Quatermann, 1987).

Dietary calcium intake also appears to affect Pb absorption. An inverse relationship has been observed between dietary calcium intake and blood Pb concentration in children, suggesting that children who are calcium-deficient may absorb more Pb than calcium-replete children (Mahaffey et al., 1986; Ziegler et al., 1978). An effect of calcium on Pb absorption is also evident in adults. In experimental studies of adults, absorption of a single dose of Pb (100 to 300 µg Pb chloride) was lower when the Pb was ingested together with calcium carbonate (0.2 g calcium carbonate) than when the Pb was ingested without additional calcium (Blake and Mann, 1983; Heard and Chamberlain, 1982). A similar effect of calcium occurs in rats (Barton et al., 1978b). In other experimental animal models, Pb absorption from the GI tract has been shown to be enhanced by dietary calcium depletion or administration of vitamin D (Mykkänen and Wasserman, 1981, 1982).

Effect of Pregnancy. Absorption of Pb may increase during pregnancy. Although there is no direct evidence for this in humans, an increase in Pb absorption may contribute, along with other mechanisms (e.g., increased mobilization of bone Pb), to the increase in blood Pb concentration observed during the later half of pregnancy (Gulson et al., 1997, 1998b, 2004; Lagerkvist et al., 1996; Rothenberg et al., 1994; Schuhmacher et al., 1996).

Effect of Dose. Lead absorption in humans may be a capacity-limited process, in which case the percentage of ingested Pb that is absorbed may decrease with increasing rate of Pb intake. However, available studies to date do not provide a firm basis for discerning if the GI absorption of Pb is limited by dose. Numerous observations of nonlinear relationships between blood Pb concentration and Pb intake in humans provide support for the likely existence of a saturable absorption mechanism or some other capacity-limited process in the distribution of Pb in humans (Pocock et al., 1983; Sherlock et al., 1982, 1984; Sherlock and Quinn, 1986).

However, in immature swine that received oral doses of Pb in soil, Pb dose-blood Pb relationships were curvilinear, whereas dose-tissue Pb relationships for bone, kidney, and liver were linear (Casteel et al., 2006). The same pattern (nonlinearity for blood Pb concentration and linearity for tissues) was observed in swine administered Pb acetate intravenously (Casteel et al., 1997). These results suggest that the nonlinearity in the Pb dose-blood Pb concentration relationship may derive from an effect of Pb dose on some aspect of the biokinetics of Pb other than absorption (e.g., saturation of binding of Pb in red blood cells). In fasted rats, absorption was estimated at 42 and 2% following single oral administration of 1 and 100 mg Pb/kg, respectively, as Pb acetate, suggesting a limitation on absorption imposed by dose (Aungst et al., 1981). Saturable mechanisms of absorption have been inferred from measurements of net flux kinetics of Pb in the in situ-perfused mouse intestine, the in situ-ligated chicken intestine, and the in vitro-isolated segments of rat intestine (Aungst and Fung, 1981; Barton, 1984; Flanagan et al., 1979; Mykkänen and Wasserman, 1981). While evidence for capacity-limited processes at the level of the intestinal epithelium is compelling, the dose at which absorption becomes appreciably limited in humans is not known.

Effect of Particle Size. Particle size influences the degree of GI absorption (Ruby et al., 1999). In rats, an inverse relationship was found between absorption and particle size of Pb in diets containing metallic Pb particles that were ≤250 µm in diameter (Barltrop and Meek, 1979). Tissue Pb concentration was a 2.3-fold higher when rats ingested an acute dose (37.5 mg Pb/kg) of Pb particles <38 µm in diameter than when rats ingested particles having diameters in the range of 150 to 250 µm (Barltrop and Meek, 1979). Dissolution kinetics experiments with Pb-bearing mine waste soil suggest that surface area effects control dissolution rates for particles sizes of <90 µm diameter; however, dissolution of 90- to 250-µm particle size fractions appeared to be controlled more by surface morphology (Davis et al., 1994). Similarly, Healy et al. (1992) found that the solubility of Pb sulfide in gastric acid in vitro was much greater for particles 30 µm in diameter than for particles 100 µm in diameter.

Absorption from Soil. Lead in soil can exist in a variety of mineralogical contexts, which can affect Pb solubility in the GI tract and, potentially, Pb absorption from the GI tract. In adult subjects who ingested soil (particle size <250 µm) collected from the Bunker Hill NPL site, 26% (SD 8.1) of the resulting 250-µg/70-kg body weight Pb dose was absorbed when the soil was ingested in the fasted state, and 2.5% (SD 1.7) was absorbed when the same soil Pb dose

was ingested with a meal (Maddaloni et al., 1998). The dominant Pb minerals in the sample (relative Pb mass) contained Pb oxides (~40%), Pb sulfates (~25%), and Pb sulfides (~11%). Absorption reported for fasted subjects (26%) was approximately half that reported for soluble Pb ingested by fasting adults, i.e., ~60% (Blake et al., 1983; Heard and Chamberlain, 1983; James et al., 1985; Rabinowitz et al., 1980). Measurements of the absorption of ingested soil Pb in infants or children have not been reported.

Relative bioavailability (RBA) of Pb in soils (i.e., ratio of estimated absorbed fraction of ingested soil Pb to that of a water-soluble form of Pb, based on measurements of ingested Pb recovered in blood and/or other tissues) has been more extensively studied in animal models. The gastric function of juvenile swine is thought to be sufficiently similar to that of human children to justify use of juvenile swine as a model for assessing RBA of Pb in soils (Casteel et al., 1996; 2006; Weis and LaVelle, 1991). In immature swine that received oral doses of soil-like materials from various mine waste sites (75- or 225-µg Pb/kg body weight), relative bioavailability of soil-borne Pb ranged from 6 to 100% compared to that of a similar dose of highly water-soluble Pb acetate (Figure 4-1; Casteel et al., 2006). Electron microprobe analyses of Pb-bearing grains in the various test materials revealed that the grains ranged from as small as 1 to 2 µm up to a maximum of 250 µm (the sieve size used in preparation of the samples) and that the Pb was present in a wide range of different mineral associations (phases), including various oxides, sulfides, sulfates, and phosphates. These variations in size and mineral content of the Pb-bearing grains are the suspected cause of variations in the rate and extent of GI absorption of Pb from different samples of soil. Based on these very limited data, the relative oral bioavailability of Pb mineral phases were categorized into “low” (<0.25), “medium” (0.25 to 0.75), and “high” (>0.75) relative bioavailability categories (Figure 4-2; Casteel et al., 2006). Mineral phases observed in mineralogical wastes can be expected to change over time (i.e., weathering), which could change the relative bioavailability of the Pb in soils.

Studies conducted in rats also indicate that the bioavailability of Pb in soils can be lower than that of water-soluble forms of Pb (e.g., Pb acetate) and that the ingestion of soil can lower the bioavailability of water-soluble Pb (Freeman et al., 1992; 1994, 1996).

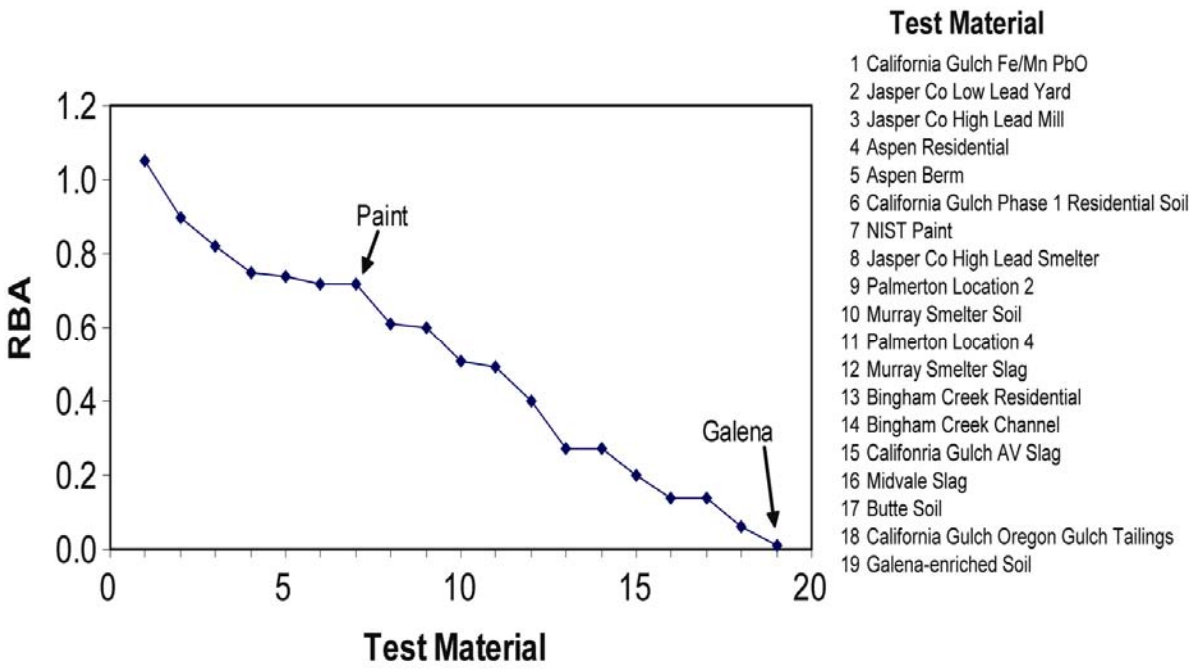

Figure 4-1. Relative bioavailability (RBA) is the bioavailability of the lead in the test material compared to that of lead acetate (test material lead acetate). The test material numbers on the horizontal axis refer to the numbered test materials in the legend.

Source: Casteel et al. (2006).

Dermal Exposure

Inorganic Lead

Dermal absorption of inorganic Pb compounds is generally considered to be much less than absorption by inhalation or oral routes of exposure; however, few studies have provided quantitative estimates of dermal absorption of inorganic Pb in humans, and the quantitative significance of the dermal absorption pathway as a contributor to Pb body burden in humans remains an uncertainty. Lead was detected in the upper layers of the stratum corneum of Pb-battery workers prior to their shifts and after cleaning of the skin surface (Sun et al., 2002), suggesting adherence and/or possible dermal penetration of Pb. Following skin application of ^{203}Pb-labeled Pb acetate in cosmetic preparations (0.12 mg Pb in 0.1 mL or 0.18 mg Pb in 0.1 g of a cream) to eight male volunteers for 12 h, absorption was ≤0.3%, based on whole-body,

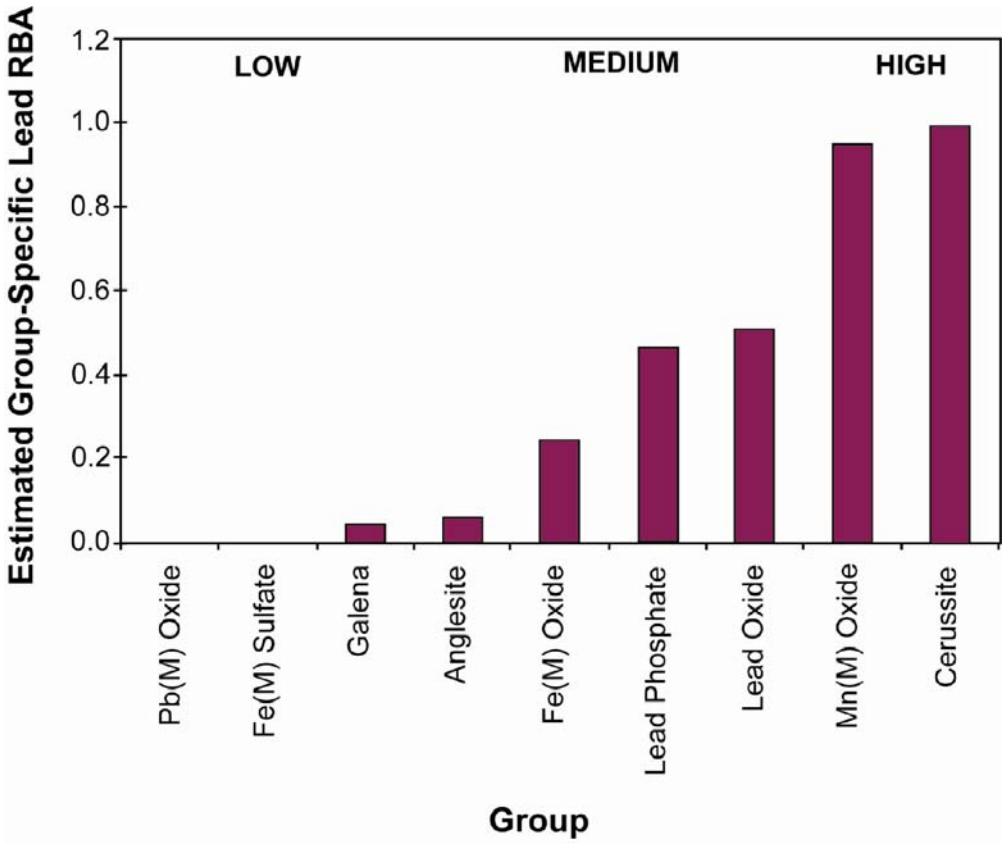

Figure 4-2. Estimated relative bioavailability (RBA, compared to lead acetate) of ingested lead in mineral groups, based on results from juvenile swine assays.

Source: Casteel et al. (2006).

urine, and blood ^{203}Pb measurements and was predicted to be 0.06% during normal use of such preparations (Moore et al., 1980). Most of the absorption took place within 12 h of exposure. Lead also appears to be absorbed across human skin when applied to the skin as Pb nitrate; however, quantitative estimates of absorption have not been reported. Lead (4.4 mg, as Pb nitrate) was applied (vehicle or solvent not reported) to an occluded filter placed on the forearm of an adult subject for 24 h, after which the patch was removed, the site cover and the forearm were rinsed with water, and total Pb was quantified in the cover material and rinse (Stauber et al., 1994). The amount of Pb recovered from the cover material and rinse was 3.1 mg (70% of the applied dose). Based on this recovery measurement, 1.3 mg (30%) of the applied dose remained

either in the skin or had been absorbed by 24 h; the amount that remained in or on the skin and the fate of this Pb (e.g., exfoliation) was not determined.

Exfoliation has been implicated as an important pathway of elimination of other metals from skin (e.g., inorganic mercury; Hursh et al., 1989). The concentrations of Pb in sweat collected from the right arm increased 4-fold following the application of Pb to the left arm, indicating that some Pb had been absorbed (amounts of sweat collected or total Pb recovered in sweat were not reported). In similar experiments with three subjects, measurements of ^{203}Pb in blood, sweat and urine, made over a 24-h period following dermal exposures to 5 mg Pb as ^{203}Pb nitrate or acetate, accounted for <1% of the applied (or adsorbed) dose. This study also reported that absorption of Pb could not be detected from measurements of Pb in sweat following dermal exposure to Pb as Pb carbonate.

Studies conducted in animals suggest that dermal penetration of inorganic Pb may vary with Pb species. Dermal absorption of Pb applied as Pb arsenate appeared to be less than of Pb acetate, based on measurements of kidney Pb levels following application of either compound to the shaved skin of rats (Laug and Kunze, 1948).

Organic Lead

Relative to inorganic Pb and organic Pb salts, tetraalkyl Pb compounds have been shown to be rapidly and extensively absorbed through the skin of rabbits and rats (Kehoe and Thamann, 1931; Laug and Kunze, 1948). A 0.75-mL amount of tetraethyl Pb, which was allowed to spread uniformly over an area of 25 cm^2 on the abdominal skin of rabbits, resulted in 10.6 mg of Pb in the carcass at 0.5 h and 4.4 mg at 6 h (Kehoe and Thamann, 1931). In a comparative study of dermal absorption of inorganic and organic salts of Pb conducted in rats, ~100 mg of Pb was applied in an occluded patch to the shaved backs of rats. Based on urinary Pb measurements made prior to and for 12 days following exposure, Pb compounds could be ranked according to the relative amounts absorbed (i.e., percent of dose recovered in urine): Pb naphthenate (0.17%), Pb nitrate (0.03%), Pb stearate (0.006%), Pb sulfate (0.006%), Pb oxide (0.005%), and metal Pb powder (0.002%). This rank order (i.e., Pb naphthalene > Pb oxide) is consistent with a rank ordering of penetration rates of inorganic and organic Pb salts through excised skin from humans and guinea pigs: Pb nuolate (Pb linoleic and oleic acid complex) > Pb naphthenate > Pb acetate > Pb oxide (nondetectable) (Bress and Bidanset, 1991).

4.2.2 Distribution

Inorganic Lead

Lead in Blood. Blood Pb concentrations vary considerably with age, physiological state (e.g., pregnancy, lactation, menopause), and numerous factors that affect exposure to Pb. Lead in blood is found primarily (~99%) in the red blood cells (Bergdahl et al., 1997b, 1998, 1999; Hernandez-Avila et al., 1998; Manton et al., 2001; Schutz et al., 1996; Smith et al., 2002). Most of the Pb found in red blood cells is bound to proteins within the cell rather than the erythrocyte membrane. ALAD is the primary binding ligand for Pb in erythrocytes (Bergdahl et al., 1997b, 1998; Sakai et al., 1982; Xie et al., 1998). Lead binding to ALAD is saturable; the binding capacity (K_{max}) has been estimated to be ~850 µg/dL for red blood cells and the apparent dissociation constant (K_d) has been estimated to be ~1.5 µg/L (Bergdahl et al., 1998). Two other Pb-binding proteins have been identified in the red cell, a 45 kDa protein (K_{max}, 700µg/dL; K_d 5.5 µg/L) and a smaller protein(s) having a molecular weight <10 kDa (Bergdahl et al., 1996, 1997b, 1998). Of the three principal Pb-binding proteins identified in red blood cells, ALAD has the strongest affinity for Pb (Bergdahl et al., 1998) and appears to dominate the ligand distribution of Pb (35 to 84% of total erythrocyte Pb) at blood Pb levels below 40 µg/dL (Bergdahl et al., 1996, 1998; Sakai et al., 1982).

Approximately 40 to 75% of Pb in the plasma is bound to plasma proteins, of which albumin appears to be the dominant ligand (Al-Modhefer et al., 1991; Ong and Lee, 1980). Lead may also bind to γ globulins (Ong and Lee, 1980). Lead in serum that is not bound to protein exists largely as complexes with low molecular weight sulfhydryl compounds (e.g., cysteine, homocysteine) and other ligands (Al-Modhefer et al., 1991). Free ionized Pb (i.e., Pb^{2+}) in plasma represents an extremely small percentage of total plasma Pb. The concentration of Pb^{2+} in fresh serum, as measured by an ion-selective Pb electrode, was reported to be 1/5,000 of the total serum Pb (Al-Modhefer et al., 1991).

Lead in Bone. In human adults, ~94% of the total body burden of Pb is found in the bones. In contrast, bone Pb accounts for 73% of the body burden in children (Barry 1975). Lead concentrations in bone and bone Pb burden (mass) increase with age throughout the lifetime, indicative of a relatively slow turnover of Pb in adult bone (Barry 1975, 1981; Gross et al., 1975; Schroeder and Tipton, 1968). The age-related changes in bone Pb concentration do not exactly correspond to the change in Pb burden as a result of skeletal growth during childhood and

adolescence, which results in a dilution of the bone Pb burden in a larger skeletal mass. The large pool of Pb in adult bone can serve to maintain blood Pb levels long after external exposure has ended (Fleming et al., 1997; Inskip et al., 1996; Kehoe, 1987; O'Flaherty et al., 1982; Smith et al., 1996). It can also serve as a source of Pb transfer to the fetus when maternal bone is resorbed for the production of the fetal skeleton (Franklin et al., 1997; Gulson et al., 1997, 1999b, 2003). See Section 4.3.2.3 for a more complete discussion of these topics.

Lead is not distributed uniformly in bone. Lead accumulates in those bone regions undergoing the most active calcification at the time of exposure. During infancy and childhood, bone calcification is most active in trabecular bone, whereas in adulthood, calcification occurs at sites of remodeling in cortical and trabecular bone. This suggests that Pb accumulation will occur predominantly in trabecular bone during childhood, but in both cortical and trabecular bone in adulthood (Aufderheide and Wittmers, 1992). A portion of Pb in mature bone is essentially inert, having an elimination half-time of several decades. A labile compartment exists as well that allows for maintenance of an equilibrium between bone and soft tissue or blood (Rabinowitz et al., 1976). Although a high bone formation rate in early childhood results in the rapid uptake of circulating Pb into mineralizing bone, bone Pb is also recycled to other tissue compartments or excreted in accordance with a high bone resorption rate (O'Flaherty 1995). Thus, most of the Pb acquired early in life is not permanently fixed in the bone (O'Flaherty, 1995). In general, bone turnover rates decrease as a function of age, resulting in slowly increasing bone Pb levels among adults (Barry, 1975; Gross et al., 1975; Schroeder and Tipton, 1968). An X-ray fluorescence study of tibial Pb concentrations in individuals older than 10 years showed a gradual increase in bone Pb after age 20 (Kosnett et al., 1994). In 60- to 70-year-old men, the total bone Pb burden may be ≥200 mg, while children <16 years old have been shown to have a total bone Pb burden of ~8 mg (Barry, 1975). However, in some bones (i.e., mid femur and pelvic bone), the increase in Pb content plateaus at middle age and then decreases at higher ages (Drasch et al., 1987). This decrease is most pronounced in females and may be due to osteoporosis and the release of Pb from resorbed bone to blood (Gulson et al., 2002). Bone Pb burdens in adults are slowly lost by diffusion (heteroionic exchange) as well as by resorption (O'Flaherty, 1995). Bone Pb stores can contribute substantially to blood Pb, and maternal bone Pb can be transferred to the fetus during pregnancy and to breast milk and nursing infants during lactation (see Sections 4.3.2.4 and 4.3.2.5 for further discussion).

Lead in Soft Tissues. Several studies have compared soft tissue concentrations of Pb in autopsy samples (Barry, 1975, 1981; Gross et al., 1975; Schroeder and Tipton, 1968). These studies were conducted in the 1960s and 1970s and, therefore, reflect burdens accrued during periods when ambient and occupational Pb exposure levels where much higher than current levels. Methods for quantifying Pb in tissues (including avoidance of contamination of the tissues) have also advanced considerably since these studies were reported. Average blood Pb concentrations reported in the adults subjects were ~20 µg/dL in the Barry (1975) and Gross et al. (1975) studies, whereas more current estimates of the average for adults in the United States are below 5 µg/dL (Centers for Disease Control and Prevention, 2005). Levels in other soft tissues also appear to have decreased substantially since these studies were reported. For example, average Pb concentrations in kidney cortex of male adults were 0.78 µg/g wet tissue and 0.79 µg/g, as reported by Barry (1975) and Gross et al. (1975), respectively (samples in the Barry study were from subjects who had no known occupational Pb exposures). In a more recent analysis of kidney biopsy samples collected in Sweden, the mean level of Pb in kidney cortex among subjects not occupationally exposed to Pb was 0.18 µg/g (maximum, 0.56 µg/g) (Barregård et al., 1999).

In spite of the downward trends in soft tissue Pb levels, the autopsy studies provide a basis for describing the relative soft tissue distribution of Pb in adults and children. Most of the Pb in soft tissue is in liver. Relative amounts of Pb in soft tissues as reported by Schroeder and Tipton (1968), expressed as percent of total soft tissue Pb, were liver, 33%; skeletal muscle, 18%; skin, 16%; dense connective tissue, 11%; fat, 6.4%; kidney, 4%; lung, 4%; aorta, 2%; and brain, 2% (other tissues were <1%). The highest soft tissue concentrations in adults also occur in liver and kidney cortex (Barry, 1975; Gerhardsson et al., 1986, 1995b; Gross et al., 1975; Oldereid et al., 1993). The relative distributions of Pb in soft tissues in males and females, expressed in terms of tissue:liver concentration ratios, were liver, 1.0 (~1 µg/g wet weight); kidney cortex, 0.8; kidney medulla, 0.5; pancreas, 0.4; ovary, 0.4; spleen, 0.3; prostate, 0.2; adrenal gland, 0.2; brain, 0.1; fat, 0.1; testis, 0.08; heart, 0.07; and skeletal muscle, 0.05 (Barry, 1975; Gross et al., 1975). In contrast to Pb in bone, which accumulates Pb with continued exposure in adulthood, concentrations in soft tissues (e.g., liver and kidney) are relatively constant in adults (Barry 1975; Treble and Thompson 1997), reflecting a faster turnover of Pb in soft tissue relative to bone.

Maternal-Fetal-Infant Transfer. The maternal:fetal blood Pb concentration ratio, based on cord blood Pb measurements, is ~0.9 (Carbone et al., 1998; Goyer, 1990; Graziano et al., 1990). In one of the larger studies of fetal blood Pb concentration, maternal and cord blood Pb concentrations were measured at delivery in 888 mother-infant pairs; the cord/maternal ratio was relatively constant, 0.93, over a blood Pb concentration range of ~3 to 40 µg/dL (Graziano et al., 1990). A study of 159 mother-infant pairs also found a relatively constant cord:maternal ratio (0.84) over a maternal blood Pb range of ~1 to 12 µg/dL (Carbone et al., 1998). As noted in the discussion of the distribution of Pb in bone, maternal bone Pb is transferred to the fetus during pregnancy and can be transferred to breast milk and nursing infants during lactation (see Sections 4.3.2.4, 4.3.2.5 for further discussion). Breast milk:maternal blood Pb concentration ratios are, in general, <0.1, although values of 0.9 have been reported (Gulson et al., 1998b).

Organic Lead

Information on the distribution of Pb in humans following exposures to organic Pb is extremely limited. One hour following 1- to 2-min inhalation exposures to ^{203}Pb tetraethyl or tetramethyl Pb (1 mg/m^3), ~50% of the ^{203}Pb body burden was associated with liver and 5% with kidney; the remaining ^{203}Pb was widely distributed throughout the body (Heard et al., 1979). The kinetics of ^{203}Pb in blood of these subjects showed an initial declining phase during the first 4 h (tetramethyl Pb) or 10 h (tetraethyl Pb) after the exposure, followed by a phase of gradual increase in blood Pb concentration that lasted for up to 500 h after the exposure. Radioactive Pb in blood was highly volatile immediately after the exposure and transitioned to a nonvolatile state thereafter. These observations may reflect an early distribution of organic Pb from the respiratory tract, followed by a redistribution of dealkylated Pb compounds.

In a man and woman who accidentally inhaled a solvent containing 31% tetraethyl Pb (17.6% Pb by weight), Pb concentrations in the tissues, from highest to lowest, were liver, kidney, brain, pancreas, muscle, and heart (Bolanowska et al., 1967). In another incident, a man ingested a chemical containing 59% tetraethyl Pb (38% Pb w/w); the Pb concentration was highest in the liver followed by kidney, pancreas, brain, and heart (Bolanowska et al., 1967).

4.2.3 Metabolism

Inorganic Lead

Metabolism of inorganic Pb consists of formation of complexes with a variety of protein and nonprotein ligands. Major extracellular ligands include albumen and nonprotein sulfhydryls. The major intracellular ligand in red blood cells is ALAD. Lead in other soft tissues such as kidney, liver, and brain exists predominantly bound to protein. High affinity cytosolic Pb-binding proteins (PbBPs) generally only observed in male rats have been identified in rat kidney and brain (DuVal and Fowler, 1989; Fowler, 1989). The PbBPs of rat are cleavage products of α2μ globulin, a member of the protein superfamily known as retinol-binding proteins that are generally observed only in male rats (Fowler and DuVal, 1991). Other high-affinity Pb-binding proteins (Kd ~14 nM) have been isolated in human kidney, two of which have been identified as a 5 kD peptide, thymosin 4 and a 9 kD peptide, acyl-CoA binding protein (Smith et al., 1998). Lead also binds to metallothionein, but does not appear to be a significant inducer of the protein in comparison with the inducers cadmium and zinc (Eaton et al., 1980; Waalkes and Klaassen, 1985). In vivo, only a small fraction of the Pb in the kidney is bound to metallothionein, and it appears to have a binding affinity that is less than Cd^{2+}, but higher than Zn^{2+} (Ulmer and Vallee, 1969); thus, Pb will more readily displace zinc from metallothionein than cadmium (Goering and Fowler, 1987; Nielson et al., 1985; Waalkes et al., 1984).

Organic Lead

Alkyl Pb compounds undergo oxidative dealkylation catalyzed by cytochrome P450 in liver and, possibly, in other tissues. Few studies of the metabolism of alkyl Pb compounds in humans have been reported. Occupational monitoring studies of workers who were exposed to tetraethyl Pb have shown that tetraethyl Pb is excreted in the urine as diethyl Pb, ethyl Pb, and inorganic Pb (Turlakiewicz and Chmielnicka, 1985; Vural and Duydu, 1995; Zhang et al., 1994). Trialkyl Pb metabolites were found in the liver, kidney, and brain following exposure to the tetraalkyl compounds in workers; these metabolites have also been detected in brain tissue of nonoccupational subjects (Bolanowska et al., 1967; Nielsen et al., 1978). In volunteers exposed by inhalation to 0.64 and 0.78 mg Pb/m^3 of ^{203}Pb-labeled tetraethyl and tetramethyl Pb, respectively, Pb was cleared from the blood within 10 h, followed by a reappearance of radioactivity back into the blood after ~20 h (Heard et al., 1979). The high level of radioactivity

initially in the plasma indicates the presence of tetraalkyl/trialkyl Pb. The subsequent rise in blood radioactivity, however, probably represents water-soluble inorganic Pb and trialkyl and dialkyl Pb compounds that were formed from the metabolic conversion of the volatile parent compounds (Heard et al., 1979).

4.2.4 Excretion

Inorganic Lead

The kinetics of elimination of Pb from the body reflects the existence of fast and slow pools of Pb in the body. Blood, which comprises ~1% of body burden, exchanges with both slow and fast pools and exhibits multiphasic elimination kinetics. The dominant phase, exhibited shortly after a change in exposure occurs, has a half-life of ~20 to 30 days. A slower phase becomes evident with longer observation periods following a decrease in exposure. The half-life of this slow phase has been estimated to be ~3 to 30 years and appears to correlate with finger bone Pb levels and is thought to reflect the release of Pb from bone stores to blood.

Independent of the route of exposure, absorbed Pb is excreted primarily in urine and feces; sweat, saliva, hair, nails, and breast milk are minor routes of excretion (Chamberlain et al., 1978; Griffin et al., 1975; Hursh and Suomela, 1968; Hursh et al., 1969; Kehoe, 1987; Moore et al., 1980; Rabinowitz et al., 1976; Stauber et al., 1994). Fecal excretion accounts for ~one-third of total excretion of absorbed Pb (fecal:urinary excretion ratio of ~0.5), based on intravenous injection studies conducted in humans (Chamberlain et al., 1978). A similar value for fecal:urinary excretion ratio, ~0.5, has been observed following inhalation of submicron Pb particles (Chamberlain et al., 1978; Hursh et al., 1969). Estimates of blood-to-urine clearance range from 0.03 to 0.3 L/day with a mean of 0.12 L/day (Araki et al., 1990; Berger et al., 1990; Chamberlain et al., 1978; Gulson et al., 2000; Koster et al., 1989; Manton and Malloy, 1983; Rabinowitz et al., 1973, 1976; Ryu et al., 1983; see Diamond, 1992 for an analysis of these data).

Much of the available information on the excretion of ingested Pb in adults derives from studies conducted on five male adults who received daily doses of ^{207}Pb nitrate for periods up to 210 days (Rabinowitz et al., 1976). The dietary intakes of the subjects were reduced to accommodate the tracer doses of ^{207}Pb without increasing daily intake, thus preserving a steady state with respect to total Pb intake and excretion. Total Pb intakes (diet plus tracer) ranged from ~210 to 360 µg/day. Urinary excretion accounted for ~12% of the daily intake (range for five

subjects, 7 to 17%) and fecal excretion, ~90% of the daily intake (range, 87 to 94%). Based on measurements of tracer and total Pb in saliva, gastric secretions, bile, and pancreatic secretions (samples collected from three subjects by intubation), GI secretion of Pb was estimated to be ~2.4% of intake (range, 1.9 to 3.3%). In studies conducted at higher ingestion intakes, 1 to 3 mg/day for up to 208 weeks, urinary Pb excretion accounted for ~5% of the ingested dose (Kehoe, 1987).

Organic Lead

Lead absorbed after inhalation of tetraethyl and tetramethyl Pb is excreted in exhaled air, urine, and feces (Heard et al., 1979). Following 1- to 2-min inhalation exposures to ^{203}Pb tetraethyl (1 mg/m^3), in four male subjects, 37% of inhaled ^{203}Pb was initially deposited in the respiratory tract, of which ~20% was exhaled in the subsequent 48 h (Heard et al., 1979). In a similar experiment conducted with ^{203}Pb tetramethyl Pb, 51% of the inhaled ^{203}Pb dose was initially deposited in the respiratory tract, of which ~40% was exhaled in 48 h. Lead that was not exhaled was excreted in urine and feces. Fecal:urinary excretion ratios were 1.8 following exposure to tetraethyl Pb and 1.0 following exposure to tetramethyl Pb (Heard et al., 1979). Occupational monitoring studies of workers who were exposed to tetraethyl Pb have shown that tetraethyl Pb is excreted in the urine as diethyl Pb, ethyl Pb, and inorganic Pb (Turlakiewicz and Chmielnicka, 1985; Vural and Duydu, 1995; Zhang et al., 1994).

4.3 BIOLOGICAL MARKERS OF LEAD BODY BURDENS AND EXPOSURE

4.3.1 Lead in Blood

4.3.1.1 Summary of Key Findings from the 1986 Lead AQCD

The extensive use of blood Pb concentration as a dose metric mainly reflects the greater feasibility of incorporating blood Pb measurements into clinical or epidemiologic studies, compared to other potential dose indicators such as Pb in kidney, plasma, urine, or bone (Flegal and Smith, 1995; Graziano, 1994; Skerfving, 1988). However, blood Pb measurements have several limitations as measures of Pb body burden (Mushak, 1989, 1993), as were noted in Section 13.3.2 of the 1986 Lead AQCD, which discussed attributes and limitations of blood Pb

concentration as an indicator of internal exposure. Since the 1986 Lead AQCD was completed, relevant developments include numerous studies of determinants of Pb levels in bone (see Section 4.3.2), which further support the importance of bone Pb on blood Pb as an index of Pb exposure. The enhanced understanding of Pb biokinetics has also been consolidated into exposure-biokinetics models, which not only serve to illustrate exposure-blood-body burden relationships, but also provide a means for making predictions about these relationships that can be tested experimentally or in epidemiologic studies. The basic concepts laid out in the 1986 Lead AQCD, that the concentration of Pb in blood is largely determined by the relatively recent exposure history of the individual and that it reflects the level of Pb in a relatively mobile and small compartment, remain valid. In children, who experience a more rapid turnover of bone mineral than adults, blood Pb concentrations closely parallel changes in total body burden.

4.3.1.2 Analytical Methods for Measuring Lead in Blood

Analytical methods for measuring Pb in blood include flame atomic absorption spectrometry (AAS), graphite furnace atomic absorption spectrometry (GFAAS), anode stripping voltammetry (ASV), inductively coupled plasma atomic emission spectroscopy (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS). GFAAS and ASV are generally considered to be the methods of choice (Flegal and Smith, 1995). Background correction, such as Zeeman background correction that minimizes the impact of the absorbance of molecular species, must be applied. Limits of detection for Pb using AAS are on the order of 5 to 10 µg/dL for flame AAS measurements, ~0.1 µg/dL for flameless AAS measurements, and ~0.005 µg/dL for GFAAS (Flegal and Smith, 1995; National Institute for Occupational Safety and Health, 1994). Standard methods that have been reported for blood Pb analysis are summarized in Annex Table AX4-1. Sample preparation usually consists of wet ashing in heated strong acid (National Institute for Occupational Safety and Health, 1977a,b,c,d,e); however, preparation methods not requiring wet ashing have also been reported (Aguilera de Benzo et al., 1989; Delves and Campbell, 1988; Manton and Cook, 1984; National Institute for Occupational Safety and Health, 1977f; Que Hee et al., 1985; Zhang et al., 1997). The presence of phosphate, ethylenediaminetetraacetic acid (EDTA), or oxalate can sequester Pb and cause low readings in flame AAS (National Institute for Occupational Safety and Health, 1984). A comparison of

IDMS, ASV, and GFAAS showed that all three of these methods can be used to quantify Pb levels in blood (Que Hee et al., 1985).

4.3.1.3 Levels of Lead in Blood

Blood Pb concentrations in the U.S. general population have been monitored in the National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control and Prevention. Samples for Phase 2 of NHANES III were collected during 1991 to 1994. Geometric mean blood Pb concentration of U.S. adults, ages 20 to 49 years, estimated from the NHANES III Phase 2, were 2.1 µg/dL (95% CI: 2.0, 2.2) (Pirkle et al., 1998). Among adults, blood Pb concentrations were highest in the strata that included ages 70 years and older (3.4 µg/dL; 95% CI: 3.3, 3.6). Geometric mean blood Pb concentration of children, ages 1 to 5 years, were 2.7 (95% CI: 2.5, 3.0) for the 1991 to 1994 survey period; however, the mean varied with socioeconomic status (SES) and other demographic characteristics that have been linked to Pb exposure (e.g., age of housing) (Pirkle et al., 1998). Central estimates from the NHANES III Phase 2 (1991 to 1994), when compared to those from Phase 1 of the NHANES III (1988 to 1991) and the NHANES II (1976 to 1980), indicate a downward temporal trend in blood Pb concentrations in the United States over this period. Data from the most recent survey (NHANES IV, Centers for Disease Control and Prevention, 2005) are shown in Tables 4-1 and 4-2. For survey years 2001-2002, the geometric mean blood Pb concentration for ages >1 year (n = 8,945) was 1.45 µg/dL (95% CI: 1.39, 1.52), with the geometric mean in males (n = 4,339) being 1.78 µg/dL (95% CI: 1.71, 1.86) and in females (n = 4,606) being 1.19 µg/dL (95% CI: 1.14, 1.25). Blood Pb concentrations in the U.S. general population have decreased over the past three decades as regulations regarding Pb paint, leaded fuels, and Pb-containing plumbing materials have decreased exposure. Changes in children over time are shown in Figure 4-3.

Yassin et al. (2004) analyzed occupational category strata from NHANES III (1988 to 1994; Table 4-3). The geometric mean for all adults (n = 11,126) included in the analysis was 2.42 µg/dL (GSD 6.93), with the highest means estimated for vehicle mechanics (n = 169; GM 4.80 µg/dL [GSD 3.88]) and construction workers (n = 122; GM 4.44 µg/dL [GSD 7.84]). See Annex Table AX4-2 for a summary of selected measurements of blood Pb levels in humans.

Table 4-1. Blood Lead Concentrations in United States by Age, NHANES IV (1999–2002)

Age	1–5 years		6–11 years		12–19 years		≥20 years	
Survey Period	*1999–2000*	*2001–2002*	*1999–2000*	*2001–2002*	*1999–2000*	*2001–2002*	*1999–2000*	*2001–2002*
n	723	898	909	1,044	2,135	2,231	4,207	4,772
Blood Lead (µg/dL)[a]	2.23 (1.96, 2.53)	1.70 (1.55, 1.87)	1.51 (1.36, 1.66)	1.25 (1.14, 1.36)	1.10 (1.04, 1.17)	0.94 (0.90, 0.99)	1.75 (1.68, 1.81)	1.56 (1.49, 1.62)

[a]Blood Pb concentrations presented are geometric means (95% CI).

Table 4-2. Blood Lead Concentrations in United States by Gender, NHANES IV (1999–2002)

Gender	Males		Females	
Survey Period	*1999–2000*	*2001–2002*	*1999–2000*	*2001–2002*
n	3,913	4,339	4,057	4,606
Blood Lead (µg/dL)[a]	2.01 (1.93, 2.09)	1.78 (1.71, 1.86)	1.37 (1.32, 1.43)	1.19 (1.14, 1.25)

[a]Blood Pb concentrations presented are geometric means (95% CI).

Table 4-3. Blood Lead Concentrations by Occupation, NHANES III (1988-1994)

		Blood Lead (µg/dL)		
Occupation	**n**	**GM**	**GSD**	**Maximum**
Vehicle mechanics	169	4.80	3.88	28.1
Food service workers	700	2.00	2.69	27.0
Management, professional, technical, and sales workers	4,768	2.13	4.05	39.4
Personal service workers	1,130	2.48	4.52	25.9
Agricultural workers	498	2.76	4.02	23.4
Production workers: machine operators, material movers, etc.	1,876	2.88	4.24	52.9
Laborers other than in construction	137	3.47	3.36	21.8
Transportation workers	530	3.49	5.19	22.3
Mechanics other than vehicle mechanics	227	3.50	4.91	16.6
Construction trades people	470	3.66	4.64	16.9
Construction laborers	122	4.44	7.84	36.0
Health service workers	499	1.76	2.24	22.4
All workers	**11,126**	**2.42**	**6.93**	**52.9**

Data from Yassin et al. (2004).

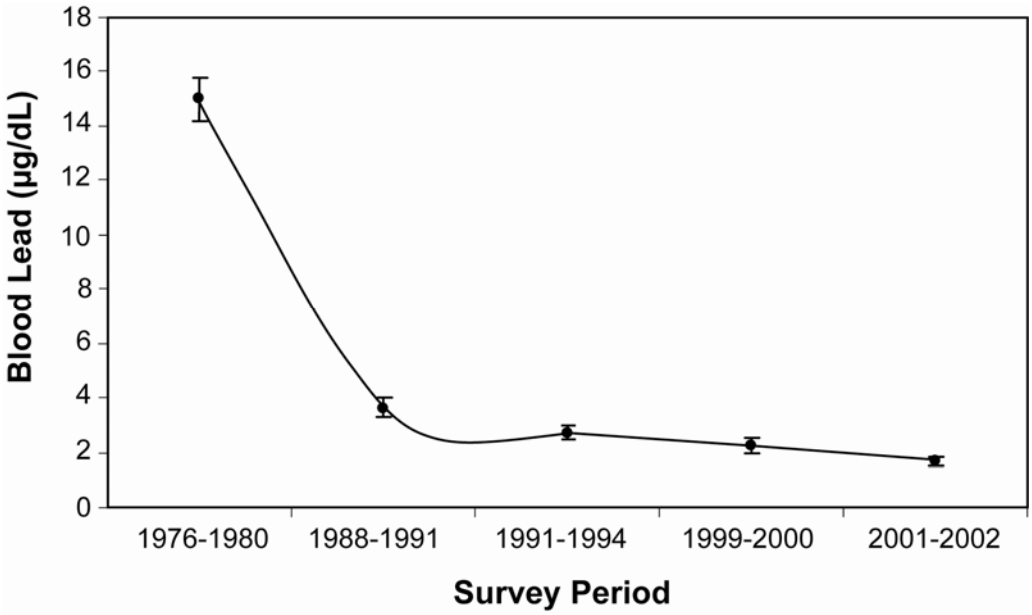

Figure 4-3. Blood lead concentrations in U.S. children, 1-5 years of age. Shown are geometric means and 95% confidence intervals as reported from the NHANES II (1976-1980) and NHANES III Phase 1 (1988-1991; Pirkle et al., 1994); NHANES III Phase 2 (1991-1994; Pirkle et al., 1998); and NHANES IV (1999-2000, 2001-2002; Centers for Disease Control and Prevention, 2005).

4.3.1.4 Blood Lead as a Biomarker of Lead Body Burden

Considerable recent effort has been directed at evaluating possible associations between Pb body burden and health outcomes, including neurodevelopmental outcomes in children (Wasserman et al., 1994) and renal/cardiovascular outcomes in adults (Cheng et al., 2001; Gerr et al., 2002; Glenn et al., 2003; Hu et al., 1996; Korrick et al., 1999; Rothenberg et al., 2002; Tsaih et al., 2004). Conceptually, measurement of long-term Pb body burden may be a preferred dose metric if the effects of Pb on a particular outcome are lasting and cumulative. However, if the effects of Pb on the outcome represent the acute effects of current exposure, then long-term body burden may not be the preferred exposure metric. In the absence of clear evidence as to which averaging time (current versus long-term) is most relevant to a particular outcome, both long-term and short-term dose metrics need be explored.

A simple conceptual representation of the Pb body burden is that it contains a fast turnover pool, comprising mainly soft tissue, and a slow pool, comprising mainly skeletal tissues (Rabinowitz et al., 1976). The rapid pool has an elimination half-life of ~28 days and comprises

<1% of the Pb body burden. The slow pool has an elimination half-life of several decades and comprises >90% of the total Pb body burden. Blood, which comprises ~1% of body burden, exchanges with both the slow and fast pools and exhibits multiphasic elimination kinetics. The dominant phase, exhibited shortly after a change in exposure occurs, has a half-life of ~20 to 30 days. A slower phase becomes evident with longer observation periods following a decrease in exposure. The half-life of this slow phase has been estimated to be ~3 to 30 years and appears to correlate with finger bone Pb levels and is thought to reflect the release of Pb from bone stores to blood. Children who have been removed from a relatively brief exposure to elevated environmental Pb exhibit faster slow-phase kinetics than children removed from exposures that lasted several years, with half-times of ~9 and ~30 months, respectively (Manton et al., 2000). This characterization is supported by measurements of Pb content of cadaver tissues (Barry, 1975; Schroeder and Tipton, 1968), Pb isotope and stable Pb kinetics in adults (Chamberlain et al., 1978; Rabinowitz et al., 1976; Griffin et al., 1975), and measurements of blood and bone Pb levels in retired Pb workers (Schütz et al., 1987a; Christoffersson et al., 1986).

As a consequence of a relatively large fraction of the body burden having a relatively slow turnover compared to blood, a constant Pb uptake (or constant intake and fractional absorption) gives rise to a quasi-steady state blood Pb concentration, while the body burden continues to increase, largely as a consequence of retention of Pb in bone (Figure 4-4). As a result, the contribution of blood Pb to body burden decreases over time. An abrupt change in Pb uptake gives rise to a relatively rapid change in blood Pb, to a new quasi-steady state, achieved in ~75 to 100 days (i.e., 3 to 4 times the blood elimination half-life). In the hypothetical simulation shown in Figure 4-4, body burden has approximately doubled (from 5 to 10 mg) as a result of a 5-year period of increased Pb uptake; however, the blood Pb concentration prior to and 1 year following cessation of the increased uptake has not changed (~2 μg/dL). Therefore, a single blood Pb concentration measurement, or a series of measurements taken over a short-time span, can be expected to be a relatively poor index of Pb body burden unless exposure over the lifetime and, thereby, body burden has been constant. On the other hand, an average of individual blood Pb concentrations measured over a longer period of time (long-term average blood Pb concentrations) can be expected to be a better index of body burden. In the hypothetical simulation shown in Figure 4-4, both the long-term average blood Pb concentration and the Pb body burden have approximately doubled.

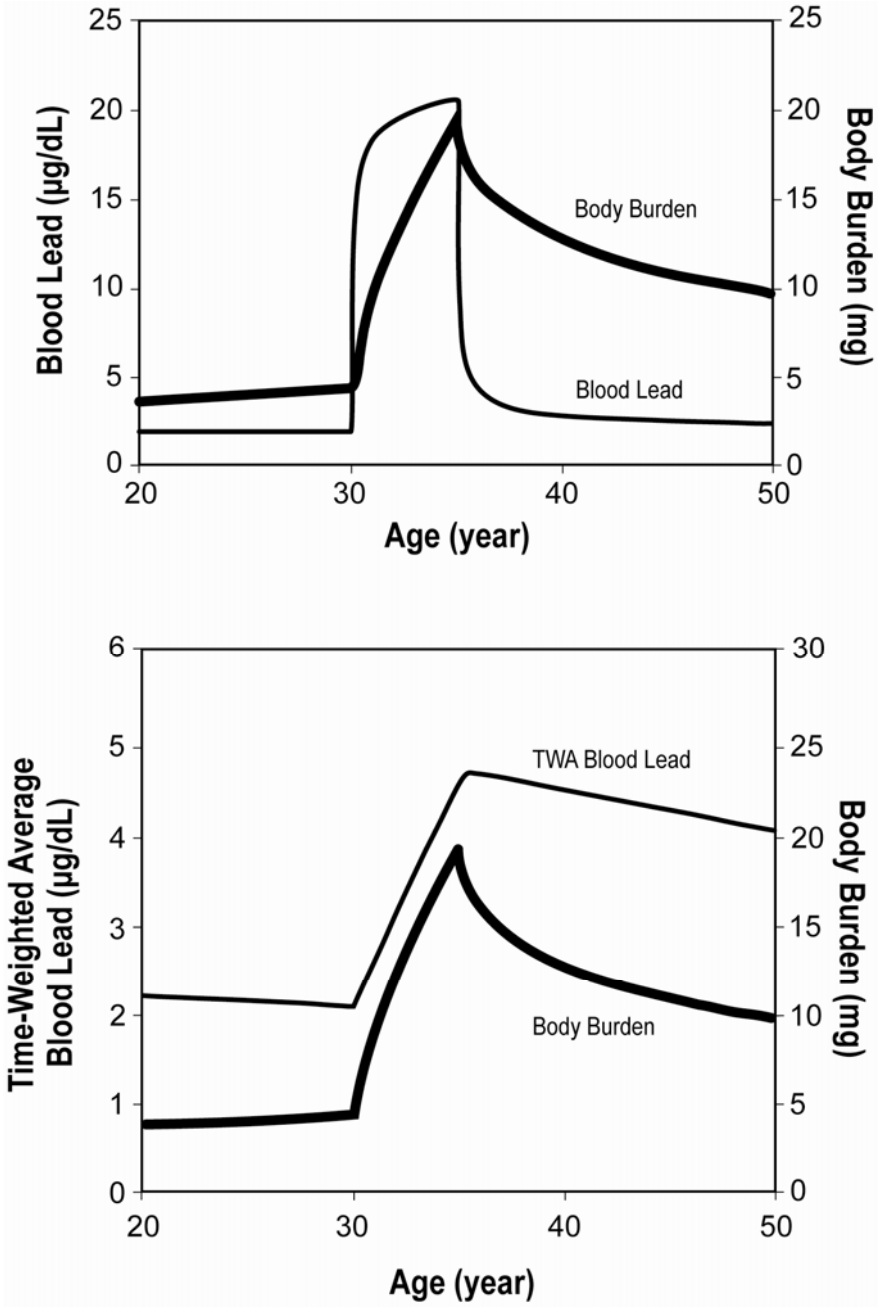

Figure 4-4. Simulation of relationship between blood lead concentration and body burden in adults. A constant baseline intake gives rise to a quasi-steady state blood lead concentration, while the body burden continues to increase, largely as a consequence of retention of lead in bone (upper panel). An abrupt change in lead uptake gives rise to a relatively rapid change in blood lead, to a new quasi-steady state, and a relatively small change in body burden. The long-term average blood lead concentration more closely tracks the pattern of change in body burden (lower panel). Simulation based on lead biokinetics model of Leggett (1993).

The disparity in the kinetics of blood Pb and body burden has important implications for the interpretation of blood Pb concentration measurements in epidemiology studies. By design, cross-sectional studies sample blood Pb concentration at one time or over relatively narrow windows of time. In these samples, the blood Pb concentration may or may not reflect well the body burden; it is more likely to do so if the measured value is a reflection of the long-term average blood Pb concentration. However, in cross-sectional samples, this cannot be ascertained. Longitudinal sampling provides a means for estimating average blood Pb concentrations over time, and such estimates are more likely to be more strongly influenced by differences in body burden than by differences in short-term variability in exposure. The degree to which repeated sampling will reflect the actual long-term time-weighted average blood Pb concentration will depend on the sampling frequency in relation to variability in exposure. High frequency variability in exposures can produce episodic (or periodic) oscillations in blood Pb concentration and body burden that may not be captured with low sampling frequencies.

The same basic concepts described above regarding Pb biokinetics of adults also apply to children. The empirical basis for the understanding of the biokinetics of Pb in children is much weaker than that for adults. However, based on the understanding of bone mineral kinetics and its importance as a mechanism for uptake and loss of Pb from bone (Leggett, 1993; O'Flaherty, 1991a,b,c, 1993, 1995), the slow pool, described above for adults, is thought to be much more labile in children, reflecting a more rapid turnover of bone mineral in children. As a result, while bone growth will contribute to accumulation of Pb in bone in children, changes in blood Pb concentration in children are thought to more closely parallel changes in total body burden (Figure 4-5). Empirical evidence in support of this comes from longitudinal studies in which relatively high correlations ($r = 0.85$) were found between concurrent ($r = 0.75$) or lifetime average blood Pb concentrations ($r = 0.85$) and tibia bone Pb concentrations (measured by XRF) in a sample of children in which average blood Pb concentrations exceeded 20 µg/dL; the correlations was much weaker ($r < 0.15$) among children who had average blood Pb concentration ≤ 10 µg/dL (Wasserman et al., 1994). Nevertheless, in children, as in adults, the long-term time-weighted average blood Pb concentration is more likely to provide a better reflection of Pb body burden than a single sample (the exception to this would be if exposure and, thereby, body burden was relatively constant throughout the lifetime).

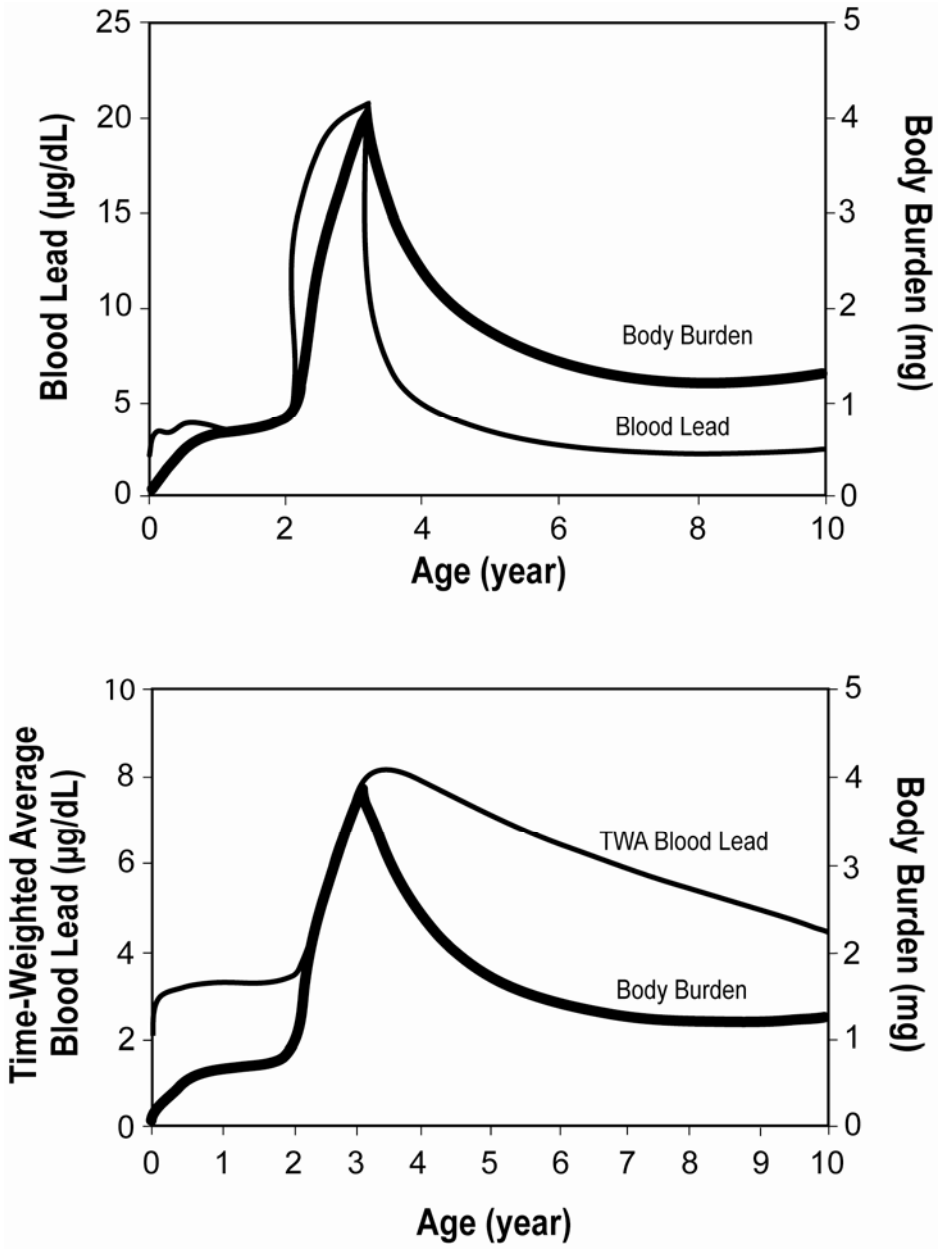

Figure 4-5. Simulation of relationship between blood lead concentration and body burden in children. Blood lead concentration is thought to parallel body burden more closely in children than in adults, due to more rapid turnover of bone and bone-lead stores in children (upper panel). Nevertheless, the long-term average blood lead concentration more closely tracks the pattern of change in body burden (lower panel). Simulation based on Leggett (1993) lead biokinetics model.

Lead that distributes from blood to tissues does so through the plasma. Therefore, theoretically, Pb concentrations in plasma should be a reflection of Pb in tissues and body burden. However, the concentration of Pb in plasma is extremely difficult to measure accurately, because levels in plasma are near the lower limits of most analytical techniques (e.g., ~0.4 µg/L at blood Pb concentration of 100 µg/L (Bergdahl and Skerfing, 1997; Bergdahl et al., 1997b), because hemolysis that occurs with typical analytical practices can contribute substantial measurement error (Bergdahl et al. 1998; Cavalleri et al. 1978; Smith et al. 1998), and because anticoagulant agents used in stabilization of blood samples for preparation of plasma can affect the distribution of Pb between blood cells and plasma (Barton, 1989; Al-Modhefer et al., 1991; Simons, 1993). Recent advances in ICP-MS offer sensitivity sufficient for measurements of Pb in plasma (Schütz et al. 1996). While the technique has been applied to assessing Pb exposures in adults (Cake et al. 1996; Hernandez-Avila et al. 1998; Manton et al. 2001; Smith et al. 2002; Tellez-Rojo et al. 2004), it has not received widespread use in epidemiologic studies.

4.3.1.5 Blood Lead as a Biomarker of Lead Exposure

Characterizing quantitative relationships between external Pb exposures and blood Pb concentrations has become central to concentration-response analyses for human populations exposed to Pb. The 1986 Lead AQCD summarized the empirical basis for this as it stood at the time. A summary of empirically-derived regression slope factors relating Pb exposures and blood Pb is provided in Abadin and Wheeler (1997). More recent meta-analyses, based on structure equation modeling, provide further support for quantitative relationships between Pb exposures and blood Pb concentrations in children (e.g., U.S. Environmental Protection Agency, 2001; Lanphear et al., 1998; Succop et al., 1998).

The elimination half-time of Pb from blood has been estimated to be ~25 to 30 days in adult males whose blood Pb concentrations were >20 µg/dL (Chamberlain et al., 1978; Rabinowitz et al., 1976; Griffin et al., 1975). In the latter studies, the elimination half-times were estimated from measurements of the time to achieve a new quasi-steady state blood Pb concentration following an increase in exposure (Griffin et al., 1975) or from measurement of the rate of change in blood concentration of an administered isotope of Pb (Chamberlain et al., 1978; Rabinowitz et al., 1976). However, the half-time for a change in blood Pb concentration (or stable isotope ratio) after an abrupt change in exposure can be much longer. Gulson et al. (1995,

1999a) estimated the half-time for the change in stable Pb isotope ratio (^{206}Pb:^{204}Pb) in blood, after an abrupt change instable isotope exposure, to be ~25 to 80 days in adult females (blood Pb concentration range 3 to 20 µg/dL). Manton et al (2000) estimated the half-time for the decline in blood Pb concentration after an abrupt decrease in exposure to be ~200 to 1000 days in children (age range: 8 to 60 months; blood Pb concentration: 7 to 5 µg/dL). The longer half-times measured under the latter conditions reflect the contribution of bone Pb stores to blood Pb following a change in exposure. Studies of the bone (or chelatable Pb) and blood Pb kinetics in retired Pb workers also demonstrate a slow phase to the elimination kinetics of blood Pb that reflects the continuing redistribution of Pb from bone to blood (Alessio, 1988; Nilsson et al., 1991; Schütz et al, 1987a; Christoffersson et al., 1986).

Based on these observations, a single blood Pb concentration may reflect the near-term or longer-term history of the individual to varying degrees, depending on the relative contributions of internal (e.g., bone) and external sources of Pb to blood Pb, which in turn will depend on the exposure history and possibly age-related characteristics of bone turnover.

Analyses of serial blood Pb concentrations measured in longitudinal epidemiologic studies have found relatively strong correlations (e.g., r = 0.5 to 0.8) between individual blood Pb concentrations measured after 6 to 12 months of age (Dietrich et al., 1993; McMichael et al., 1988; Otto et al., 1985; Rabinowitz et al., 1984; Schnaas et al, 2000). These observations suggest that, in general, exposure characteristics of an individual child (e.g., exposure levels and/or exposure behaviors) tend to be relatively constant across age. However, a single blood Pb measurement may not distinguish between a history of long-term lower-level Pb exposure from a history that includes higher acute exposures (Mushak, 1998). This is illustrated in Figure 4-6. Two hypothetical children are simulated. Child A has a relatively constant Pb intake from birth, whereas Child B has the same long-term Pb intake as Child A but with a 1-year elevated intake beginning at age 24 months (Figure 4-6, upper panel). The absorption fraction is assumed to be the same for both children. Blood Pb samples 1 and 5, or 2 and 4, will yield similar blood Pb concentrations (~3 or 10 µg/dL, respectively), yet the exposure contexts for these samples are very different. Two samples (e.g., 1 and 2, or 4 and 5), at a minimum, are needed to ascertain if the blood Pb concentration is changing over time. The rate of change can provide information about the magnitude of change in exposure, but not necessarily about the time history of the change (Figure 4-6, lower panel). Here again, time-integrated measurements of Pb concentration

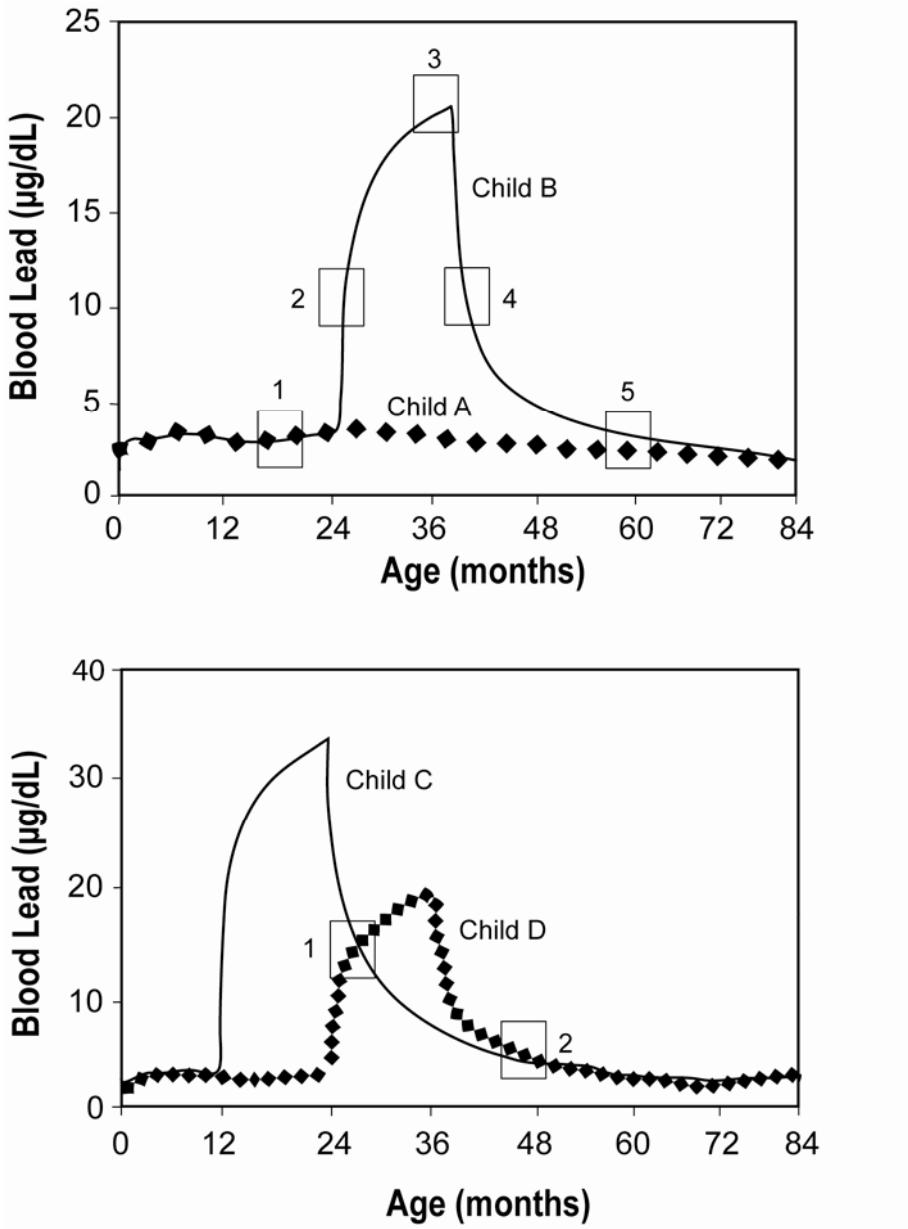

Figure 4-6. Simulation of temporal relationships between lead exposure and blood lead concentration in children. Child A and Child B have a relatively constant basal lead intake (µg/day/kg body weight) from birth; Child B experiences 1-year elevated intake beginning at age 24 months (upper panel). Blood lead samples 1 and 5, or 2 and 4, will yield similar blood lead concentrations (~3 or 10 µg/dL, respectively), yet the exposure scenarios for these samples are very different. As shown in the example of Child C and Child D, two samples can provide information about the magnitude of change in exposure, but not necessarily the temporal history of the change (lower panel).

may provide a means for accounting for some of these factors and, thereby, provide a better measure of long-term Pb exposure. The same concepts apply to estimation of long-term exposure based on blood Pb measurements in adults (Gerhardsson et al., 1992, 1995a; Roels et al., 1995).

An additional complication is that the relationship between Pb intake and blood Pb concentration is curvilinear, i.e., the increment in blood Pb concentration per unit of Pb intake decreases with increasing blood Pb concentration, both in children (Lacey et al., 1985; Ryu et al., 1983; Sherlock and Quinn, 1986) and in adults (Kehoe, 1987; Laxen et al., 1987; Pocock et al., 1983; Sherlock et al., 1982, 1984). The nonlinearity is evident even at blood Pb concentrations below 25 μg/dL (Figure 4-7). The nonlinearity in the Pb intake-blood Pb concentration relationship is derived, at least in part, from a capacity limitation in the accumulation of Pb in erythrocytes (Bergdahl et al., 1997a, 1998, 1999; Manton et al., 2001; Smith et al., 2002). A capacity-limited process may also reside at the level of intestinal absorption; however, the dose at which absorption becomes appreciably limited in humans is not known. Lead intake-blood Pb relationships also vary (a) with age, as a result of age-dependency of GI absorption of Pb, and (b) with diet and nutritional status (Mushak, 1991).

The blood Pb concentration is also influenced by Pb in bone. Evidence for the exchange of bone Pb and soft tissue Pb stores comes from analyses of stable Pb isotope signatures of Pb in bone and blood. As noted earlier, bone Pb likely contributes to the slow phase of elimination of Pb from blood that has been observed in retired Pb workers (Christoffersson et al., 1986; Schütz et al., 1987a). Bone Pb stores may contribute 40 to 70% of the Pb in blood (Manton, 1985; Gulson et al., 1995; Smith et al., 1996). This contribution increases during pregnancy, when mobilization of bone Pb increases, apparently as the bone is resorbed to produce the fetal skeleton (Gulson et al., 2003). The mobilization of bone Pb during pregnancy may contribute, along with other mechanisms (e.g., increased absorption), to the increase in Pb concentration that has been observed during the later stages of pregnancy (Gulson et al., 1997; Lagerkvist et al., 1996; Schuhmacher et al., 1996). In addition to pregnancy, other states of increased bone resorption appear to result in release of bone Pb to blood; these include lactation, osteoporosis, and menopause (Gulson et al., 2003). These observations are consistent with epidemiologic studies that have shown increases in blood Pb concentration after menopause and in association with decreasing bone density in postmenopausal women (Hernandez-Avila et al., 2000;

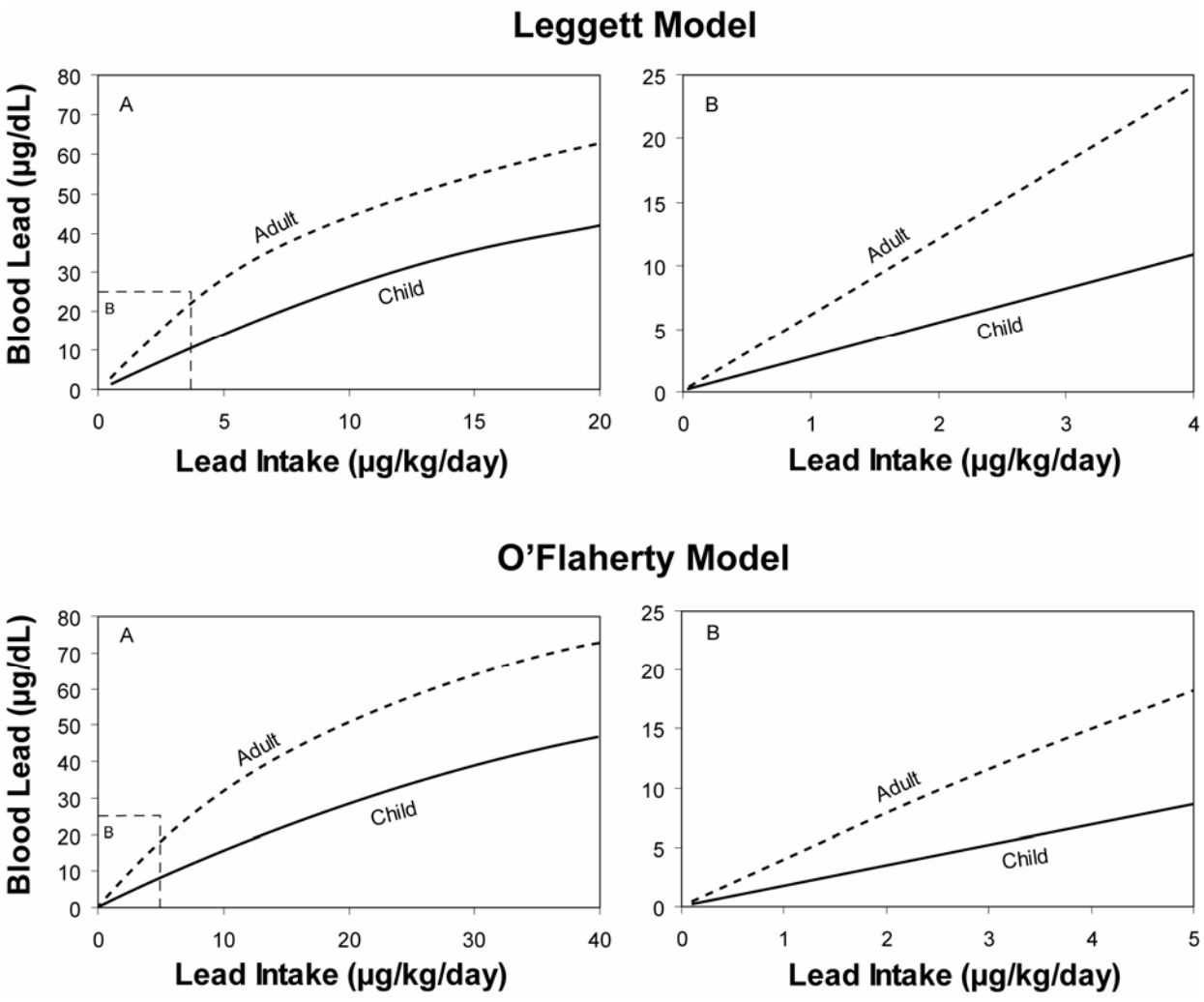

Figure 4-7. Simulation of relationships between lead intake and blood lead concentration in adults and children. The relationship between lead intake and blood lead concentration is curvilinear in adults and children. Predictions are for a 2-year-old child and 30-year-old adult, for a constant lead intake (µg/kg/day). Predictions are based on Leggett (1993, upper panel) and O'Flaherty (1993, 1995, lower panel). Right panels provide an enlarged view of lower intakes for each model.

Nash et al., 2004; Symanski and Hertz-Picciotto, 1995). The relationship between blood and bone Pb is discussed further in Section 4.3.2 on bone Pb as a biomarker of Pb exposure.

4.3.1.6 Summary of Blood Lead as a Biomarker of Lead Body Burden and Exposure

The blood Pb concentration measured in an individual will be determined by the recent exposure history of the individual as well as by the long-term exposure history that gives rise to accumulated bone Pb stores. The contribution of the latter to blood Pb may change with the duration and intensity of the exposure, age, and various physiological variables (e.g., nutritional status, pregnancy, and menopause). Longitudinal measurements of blood Pb can be expected to provide a more reliable measure of exposure history of an individual (and will more closely parallel body burden) compared to a single measurement; however, the degree to which this will apply will depend on the sampling frequency with respect to the temporal pattern of exposure.

In general, higher blood Pb concentrations can be interpreted as indicating higher exposures (or Pb uptakes); however, they do not necessarily predict appreciably higher body burdens. Similar blood Pb concentrations in two individuals (or populations) do not necessarily translate to similar body burdens or similar exposure histories.

4.3.2 Lead in Bone

4.3.2.1 Summary of Key Findings from the 1986 Lead AQCD

In the 1986 Lead AQCD, the discussion on the distribution of Pb in bone was fairly limited and mostly based on postmortem studies. The distribution between the two major compartments of cortical and trabecular bone was specifically addressed based on the pioneering isotopic work of Rabinowitz et al. (1977). Estimates of the amount of Pb in bone were also provided. There was limited discussion of the half-life of Pb in bone as being on the order of several decades.

One of the major conclusions of the 1986 Lead AQCD regarding bone Pb was that the traditional view that the skeletal system was a total sink for body Pb was now giving way to the notion that there were at least several bone compartments for Pb, with different mobility profiles. The possibility of bone Pb serving as a source of long-term internal exposure was also considered.

Since 1986, the main focus of Pb in bone studies has been on occupationally exposed subjects, because of concern until more recent times about the ability to measure lower levels of Pb in bone from environmentally exposed subjects. Furthermore, most of the focus has been on

adult males, with very few studies on females and children. The newly available studies of Pb in bone are discussed in the following sections.

4.3.2.2 Methodology of Bone Lead Analysis

Analytical Methods for Measuring Lead in Bone

Bone is comprised of two main types (cortical and trabecular) that have distinct rates of turnover and Pb release, resulting in potential differences in implications with respect to toxicity aspects (further discussed in Section 4.3.2.3). The most commonly measured bones are the tibia, calcaneus, patella, and finger bone. For cortical bone, the midpoint of the tibia is measured. For trabecular bone, both the patella and calcaneus are measured. Recent studies favor measurement of the patella, because it has more bone mass and may afford better measurement precision than the calcaneus. The advantages and disadvantages of patella and calcaneus sites have not been thoroughly investigated. Bone Pb measurements in cadavers, environmentally exposed subjects, and occupationally exposed subjects are presented in Annex Tables AX4-3, AX4-4, and AX4-5, respectively.

Bone analysis methods for in vivo measurements have included AAS, ASV, ICP-AES, ICP-MS, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), thermal ionization mass spectrometry (TIMS), synchrotron radiation induced X-ray emission (SRIXE), particle induced X-ray emission (PIXE), and X-ray fluorescence (XRF). Since the 1986 Lead AQCD, there have been many new papers published on bone Pb using XRF. The upsurge in popularity of the XRF method has paralleled a decline in the use of the other methods.

In the past, two main approaches for XRF measurements have been used to measure Pb concentrations in bone, the K-shell and L-shell methods. The K-shell method is now the most widely used, as there have been no further developments in L-shell devices since the early 1990s. The K-shell methods using ^{57}Cd and ^{109}Cd have been described in detail by Somervaille et al. (1989). Briefly, the K-shell XRF method uses 88.034 keV gamma rays from ^{109}Cd to fluoresce the K-shell X-rays of Pb.

Plaster-of-Paris “phantoms” with varying Pb concentrations measured by ICP-MS or AAS are used to calibrate the K-shell systems. Differences and corrections in the use of the phantoms have been discussed in, for example, Gordon et al. (1994), Kondrashov and Rothenberg (2001a), Todd et al. (2000), Todd and Chettle (2003), and Chettle et al. (2003). Todd et al. (2002b) also

commented that the calibration of ^{109}Cd-based K-shell XRF equipment with standards that consist of a Pb-doped Plaster-of-Paris matrix are not valid because Plaster of Paris is not true bone matrix. The problem of contamination from various sources (external or the matrix of the Plaster-of-Paris phantom) and its impact on variance was addressed by Todd (2000).

There have been several publications focused on the calculating estimates of Pb concentrations and uncertainties for XRF measurements (Kondrashov and Rothenberg, 2001a,b; Gordon et al. 1993; Todd, 2000; Todd and Chettle, 2003; Chettle et al., 2003; Todd et al., 2003) culminating in an agreed position in 2003 for these uncertainties (Chettle et al., 2003). Todd et al. (2000) provide a detailed discussion of the influences on the variability and measurement uncertainty including repositioning, sample measurement duration, overlying tissue, operator expertise, detector resolution, and changes to measurement process over time. Some of these aspects were also discussed by Hu et al. (1995). In a K-shell XRF study of 210 children aged 11 to 12½ years from a smelter town in Yugoslavia, Todd et al. (2001) (ER) decided that the methodological uncertainty in children was comparable to that in adults.

Apart from the recent study of the L-shell method by Todd et al. (2002a), there have been several investigations of the reproducibility and accuracy of the K-shell method. The main approaches have been repeated measurements on the same individuals within a limited time frame, repeated measurements on the same individuals over an extended time frame, repeated measurements of cadaver legs at the same location (and compared with AAS analyses), and extended measuring times (Somervaille et al., 1986; Aro et al., 2000; Gordon et al., 1994; Hoppin et al., 2000; Todd et al., 2000). In one of the earliest validation studies, Somervaille et al. (1986) compared K-shell and AAS measurements of 30 dissected tibia whose Pb values ranged from 6.5 to 83 µg/g of ashed bone. They found no evidence of a systematic difference between the two measurement techniques of more than 1 µg/g.

Short-term variability of XRF results was investigated in two recent studies of cadaver legs. In the first study, Aro et al (2000) compared Pb levels in 8 cadaver legs: XRF measures of intact bone with skin and hair, bare bones, and then ashed bone by ICP-MS. The XRF measurements involved 10 consecutive 30-min measurements on each bone. In the tibia, Pb concentrations by XRF showed standard deviations for each bone ranging from 6 to 58% for intact bone (mean 27%) and 13 to 36% for bare bone. Patella Pb concentrations had standard deviations ranging from 9 to 88% (mean 36%) for intact bone and 6 to 64% (mean 19%) for bare

bone. ANOVA results showed that after controlling for sampling variation contributed by age, gender and leg sections, no significant difference was found for mean Pb concentrations measured on intact bone and bare bone and by ICP-MS methods.

In the other cadaver study, Todd et al. (2000) measured 10 intact adult legs and the bare tibiae dissected from 9 of those legs for investigation of the short-term variability in XRF results. Each bare tibia was measured 6 times over 3 hours (without repositioning the tibia measurements) at the same location as when measured intact. In addition, 4 volunteers underwent monthly single measurements of the left tibia for 1 year. As a further check for reproducibility, half-hour measurements on the bone standard, NIST 1400, were measured: 30 measurements were taken over a period of 4 days repositioning the sample between each measurement, and 60 consecutive measurements were taken over a 30-h period without repositioning the sample. They concluded that "the uncertainty in an individual measurement is an underestimate of the standard deviation of replicate measurements, suggesting a methodological deficiency probably shared by most current ^{109}Cd-based K-shell XRF Pb measurement systems."

As part of the same study of cadaver legs, 9 tibia were divided into cross-sectional segments 2 cm apart, which were further separated into the tibia core and surface samples for the AAS measurements (Todd et al. 2002b). The authors found no statistically significant difference between mean XRF-measured concentrations and mean surface Pb concentrations measured by AAS, but the XRF-measurements for tibia core Pb concentrations were significantly overestimated by between 5 and 8 μg/g bone mineral. That is, XRF more closely reflects Pb concentrations at tibia surface than in tibia core.

Another aspect of the cadaver studies of Todd et al. (2001) measured multiple locations on the 10 intact legs and on the 9 bare tibiae. For example, each intact leg underwent single XRF measurements at each of 10 locations along a middle track, extending to 10 cm above and below the vertical midpoint of the tibia. Each of the 9 bare tibia underwent at least 6 XRF measurements without repositioning the tibia between measurements at each centimeter location on a 9 cm × 3 cm grid that covered the upper half of the tibia (27 locations). In bare tibia, mean XRF results increased up and down from the vertical midpoint of the tibia, consistent with the idea that the ends of tibia contain a larger component of trabecular bone than the middle section, thereby increasing the ^{109}Cd-based XRF result. This finding contrasted with those of Hoppin

et al. (2000) and Todd et al. (2001) attributed the difference to the smaller range of vertical displacement (±1 cm) in 4 bare bones measured by Hoppin et al. (2000). However, in single XRF spectra taken at multiple locations on intact limbs, there was no detectable effect of vertical location on XRF result; Todd et al. (2000) suggested that measuring away from the vertical midpoint of the tibia should not be of practical concern when performing in vivo XRF measurements. Aro et al. (2000) also concluded that bone Pb was reasonably homogeneously distributed.

A comparison of Pb concentrations between right and left tibia in 12 human skeletons to assess natural homogeneity of Pb content revealed natural symmetry with a calculated correlation coefficient of 0.9 (Wittmers et al., 1988). K-shell XRF measurements on left and right tibiae in 14 subjects showed no significant differences between legs (Gordon et al., 1994).

The reproducibility of XRF measurements over extended intervals has been investigated in several studies. Gordon et al. (1994) measured 5 subjects five times on two occasions 10 months apart and found mean standard deviations of 3.4 and 5.1 µg/g bone mineral for males and females, respectively. Armstrong et al. (1992) measured tibia Pb concentrations on two occasions separated by 5 years for a group of workers occupationally exposed to Pb. In 1983, the average uncertainty in a single measurement was 9.3 µg/g bone mineral with a mean Pb concentration in 15 subjects of 54.4 µg/g bone mineral. In 1988, the uncertainty was 4.9 µg/g and the mean value for 11 subjects was 44.2 µg/g. They suggested that the difference in measurements separated by 5 years could be accounted for by counting statistics. Todd et al. (2000) performed 27 replicate measurements on 10 intact cadaver legs on the same location over a period of 4½ months. The found the average difference between the (average) XRF results from short term and longer term measurements was 1.2 µg/g "showing there is a reassuringly small amount of variability in the XRF results over a sustained period of time" (p. 3743). They also performed monthly measurements on 4 adult volunteers (2 male, 2 female) over 1 year. Tibia Pb varied from 6.4 to 12.9 µg/g bone mineral and standard deviations of the measurements ranged from 4.9 to 9.9 µg/g.

Attenuation of X-rays by skin and hair can affect the bone Pb measurements. For example, normalization of the Pb X-rays to the elastic scatter was considered to render the accuracy of measurements insensitive to variations in overlying tissue thickness (Chettle et al., 1991; Hu et al., 1995). Todd et al. (2000, p 3737) state that: "The principal factor influencing a

human subject's bone Pb measurement uncertainty is the thickness of tissue overlying the bone that is measured" but concluded (page 3742) from the difference in average XRF results for intact and bare bone that the bone Pb measurements were qualitatively independent of the presence of overlying tissues.

However, in a study of young adults, McNeill et al. (1999) found that the measurement uncertainties were greater than uncertainties for occupationally exposed males and attributed this to inclusion of obese subjects and females in their study; uncertainties increased with bone body mass index and were poorer for females than males.

In a study of 108 former female smelter employees and 99 referents, Popovic et al. (2005) suggest that a high body mass index can give distorted values.

In the most recent study using nine leg phantoms of different soft tissue thickness, Ahmed et al. (2006) found that by increasing the overlying tissue thickness from 3.2 to 14.6 mm, there was an increase in average measurement uncertainty by a factor of 2.4 and an increase in minimum detectable limit by a factor of 2.46.

Since 1986, several investigators have reported refinements to hardware and software to improve the precision and accuracy of XRF measurements and there have been a number of investigations into the precision, accuracy and variability in XRF measurements (e.g., Aro et al., 2000; Todd et al., 2000, 2001, 2002). Todd et al. (2000) provided a detailed discussion of factors that influence the variability and measurement uncertainty, including repositioning, sample measurement duration, overlying tissue, operator expertise, detector resolution, and changes to measurement process over time. Some of these aspects were also discussed by Hu et al. (1995). From their cadaver and in vivo measurements, Todd et al. (2000) concluded that the uncertainty in an individual measurement was an underestimate of the standard deviation of replicate measurements, suggesting a methodological deficiency probably shared by most current ^{109}Cd-based K-shell XRF Pb measurement systems. In examining the reproducibility of the bone Pb measurements over a 4½ month period, Todd et al. found the average difference between the XRF results from short term and longer term measurements was 1.2 µg/g, indicating only a small amount of variability in the XRF results over a sustained period of time.

Statistical Methods for Analyzing Bone Lead Concentrations in Epidemiologic Studies

In the literature, XRF bone Pb data have typically been reported in two ways: one that involves a methodological approach to assessing the minimum detection limit and the other termed an epidemiologic approach by Rosen and Pounds (1998). In the methodological approach, a minimum detection limit is defined using various methods, including two or three times the square root of the background counts; one, two, or three times the SD of the background; and two times the observed median error. This approach relies upon the minimum detection limit to define a quantitative estimate that is of sufficient precision to be included in the statistical analysis. The following are examples of methodological minimum detection limits for bone Pb analyses. Bellinger et al. (1994) observed minimum detection limits, equivalent to the SD, of 5.4 μg/g for tibia and 9.2 μg/g for patella. Using twice the median observed error, Gerhardsson et al. (1993) observed minimum detection limits of 9.8 μg/g for tibia and 19.1 μg/g for calcaneus. For finger bone Pb measurements, Christoffersson et al. (1986) observed a minimum detectable limit of 20 μg/g, which was equivalent to three times the square root of the background counts.

With the epidemiologic approach, to determine the minimum detection limit of an instrument, all values are used (including negative values)—which results in extremely low detection limits. Rosen and Pounds (1998) noted that this approach yields population bone Pb averages that they considered artificially low and inconsistent with observations from many other earlier studies. However, not including values that are negative or below the detection limit, or assigning these values a fixed number for the statistical analysis is also of concern. To examine and compare the two methods used to analyze data at low levels of bone Pb concentration, Kim et al. (1995) performed serial measurements on phantoms containing spiked amounts of Pb. The results demonstrated that the use of methodological minimum detection limits to recode low-level observations reduced the efficiency of the analysis and the ability to distinguish between the phantoms. Using the epidemiologic approach of retaining all point estimates of measured bone Pb concentrations provided less bias and greater efficiency in comparing the mean or median levels of bone Pb of different populations.

4.3.2.3 Bone Lead as a Biomarker of Lead Body Burden

Uptake of Lead in Bone

The dominant compartment for Pb in the body is in bones. In human adults, more than 90% of the total body burden of Pb is found in the bones, whereas bone Pb accounts for ~70% of the body burden in children (Barry, 1975). Bone is comprised of two main types, cortical and trabecular. The tibia consists of more than 95% cortical bone, the calcaneus and patella comprise more than 95% trabecular bone, and finger bone is a mixed cortical and trabecular bone although the second phalanx is dominantly cortical. The cortical and trabecular bones have distinct rates of turnover and Pb release, as well as potentially different associated toxicity implications (Hu et al., 1998). For example, adult tibia has a turnover rate of about 2% per year whereas trabecular bone has a turnover rate of more than 8% per year (Rabinowitz, 1991). The proportion of cortical to trabecular bone in the human body varies by age, but on average is about 80 to 20 (International Commission on Radiological Protection, 1973). Although not so important for certain types of measurements, the periosteum is of limited dimension and may reflect a bone compartment of more rapid deposition and turnover of Pb than the other two types (Skerfving et al., 1993), which would also likely have implications for toxicity, especially for chelation therapy.

Much of the understanding of bone structure and metal deposition comes from studies of radioactive elements (e.g., International Commission on Radiological Protection, 1996). Durbin (1992, page 823) suggests that there is "an initial deposition of Pb on anatomical bone surfaces with some skewing to the well nourished trabecular surfaces in red marrow, intense deposits at bone growth sites, and later on, a nearly diffuse labeling throughout the bone volume.
For constant intake of Pb during growth, it is expected that Pb will be nearly uniformly distributed in the mineralized bone. Single or irregular intakes during growth are expected to result in residual buried lines and hotspots superimposed on a relatively uniform diffuse concentration in bone mineral volume. For example, periosteal and subperiosteal Pb deposits in the long bones, including those of the hands and feet, are likely to be greater than at many other sites, since bone growth continues at the periosteal surface while the endosteal surface is resorbed."

The importance of bone marrow was also stressed by Salmon et al. (1999), with a key factor affecting Pb uptake into bone being the fraction of bone surface in trabecular and cortical

bone adjacent to active bone marrow. The fraction of total marrow that is red and active decreases from 100% at birth to about 32% in adulthood (Cristy, 1981). Early Pb uptake is greater in trabecular bone due to its larger surface area and higher metabolic rate. Of the total bone surface against red marrow, 76% is trabecular and 24% is cortical endosteal (Salmon et al., 1999). Bone marrow has much lower Pb concentrations than bone matrix (Skerfving et al., 1983).

Half-life of Lead in Bone

Estimates of the half-life of Pb in trabecular bone are partly dependent on the tissue analyzed and the "purity" of the trabecular component (e.g., patella, calcaneus, and phalanx). Earlier estimates of the half-life of Pb in trabecular bone ranged from 12 to 19 years (Bergdahl et al., 1998; Gerhardsson et al., 1993). For cortical bone, estimates for the half-life of Pb were on the order of 13 to 27 years (Bergdahl et al., 1998; Gerhardsson et al., 1993; Rabinowitz, 1991).

With respect to half-lives in bone, recent K-shell XRF bone studies have indicated that earlier concepts of a constant rate of removal of Pb from bone throughout adulthood assumed in models of human metabolism (Leggett, 1993; O'Flaherty, 1993) may be incorrect. In a study of active and retired smelter workers, Brito et al. (2001) suggested that people less than 40 years old had a shorter half-life for the release of Pb from the tibia than those older than 40 years, 4.9 years (95% CI: 3.6, 7.8) compared to 13.8 years (95% CI: 9.7, 23.8), respectively. Also, they suggested that less intensely exposed subjects with a lifetime averaged blood Pb of ≤25 µg/dL had a shorter half-life in the tibia (6.2 years [95% CI: 4.7, 9.0]) than those with a lifetime averaged blood Pb >25 µg/dL (14.7 years [95% CI: 9.7, 29.9]).

Even by the end of the sixth decade, ~35 to 40% of skeletal mass consists of unremodelled first generation bone acquired during childhood and adolescence (International Commission on Radiological Protection, 1973). This statement contrasts with that of O'Flaherty (1993) who suggested that because of the relatively short half-life of Pb in the bones of children that much of the Pb incorporated during active growth would not persist into adulthood. In a comparison of Pb in tooth dentine and the tibia from young adults who were followed up after a period of 13 years, Kim et al. (1996) suggested that "pockets" of Pb acquired in childhood may persist into adults. Likewise, McNeill et al. (2000) compared tibia Pb levels and cumulative

blood Pb indices in a population of 19 to 29 year olds who had been highly exposed to Pb in childhood from the Bunker Hill, Idaho smelter. They concluded that Pb from exposure in early childhood had persisted in the bone matrix until adulthood.

Changes in Bone Lead Concentrations with Age

Conventional and XRF analyses of bone have shown significant increases in bone Pb with age (Hu et al., 1990, 1996; Kosnett et al., 1994; Morgan et al., 1990). Kosnett et al. (1994) observed no significant change in bone Pb concentrations up to age 20 years, but found an increasing trend with the same slope for men and women between the ages of 20 to 55 years and an increase to a faster rate in men older than 55 years. Kosnett et al. reanalyzed earlier cadaver cortical bone data of Drasch et al. (1987) and found that male bone Pb values increased significantly after age 40 years, whereas female values slightly declined. A similar analysis of the post-mortem data of Barry (1975) showed an upward inflection for all males after age 35 years. Kosnett et al. (1994) found no significant slope to the relationship between age and bone Pb for the 10 to 20 year old subjects, in contrast to Barry (1975) and Drasch et al. (1987). Kosnett et al. (1994) further noted that relatively high environmental Pb levels characterized various populations in the past and would have resulted in higher levels of bone Pb deposition; a portion of the increase of bone Pb with age is thus likely attributable to an exposure cohort effect.

Annual increments of Pb to bone vary, although no attempt has been made to determine whether the differences are significant. For example, the annual increment of 0.46 μg/g bone mineral/year found by Gordon et al. (1993) was slightly lower than that found by Somervaille et al. (1989), but the difference was not significant. After age 20 years, Kosnett et al. (1994) found the annual increment to be 0.38 μg/g bone mineral/year. Hu et al. (1990) reported a value of 0.31 μg/g bone mineral/year for subjects ranging in age from 20 to 58 years. Thus, interpreting variations in bone Pb as a function of age is complex.

4.3.2.4 Distribution of Lead from Bone into Blood and Plasma

Contribution of Bone Lead to Blood Lead

Although the skeleton was recognized as a potentially significant contributor to blood Pb in the 1986 Lead AQCD, there have been several investigations using both bone Pb XRF and

stable Pb isotope methods that have helped quantify the contribution. The earlier estimation of skeletal contribution to blood Pb was 70% by Manton (1985) and ~65% ranging up to 100% by Schütz et al. (1987b). The more recent isotope studies confirmed these estimates. Using female immigrants to Australia and their children, Gulson et al. (1995, 1997, 1999a) found a mean value of 50% (range 16-73%) deriving from the skeleton. Smith et al. (1996) found a range of 40–70% in five patients who underwent total hip or knee joint replacement. Gwiazda et al. (2005) observed a range of 40-65% in two children and >90% in one child. Studies examining the bone Pb contribution to blood Pb are presented in Annex Table AX4-6.

The contribution of skeletal Pb to blood Pb was further examined in females from varying environments. In middle-aged to elderly subjects (46-74 years), an increase of 19 μg/g of Pb in tibia bone mineral was associated with an increase in blood Pb of 1.7 μg/dL, which corresponds to a 0.09 μg/dL increase in blood Pb per 1 μg/g bone mineral (Korrick et al., 2002). A study of 108 former workers at the Bunker Hill smelter in northern Idaho and 99 referents from the Spokane, WA area examined the endogenous bone Pb release rate of postmenopausal and premenopausal women (Popovic et al., 2005). The results indicated that the endogenous release rate in postmenopausal women (0.13 μg/dL per μg/g bone) was greater than the rate found in premenopausal women (0.07 μg/dL per μg/g bone). In a Mexico City study, the endogenous bone Pb release rate in postmenopausal women also was observed to be double that in premenopausal women (Garrido-Latorre et al., 2003). A change of 10 μg/g bone mineral was associated with an increase in blood Pb of 1.4 μg/dL in postmenopausal subjects, compared to an increase of 0.8 μg/dL in premenopausal women. Lactation was also found to affect the endogenous bone Pb release rate. After adjusting for patella Pb concentration, an increase in blood Pb levels of 12.7% (95% CI: 6.2, 19.6) was observed for women who practiced partial lactation and an increase of 18.6% (95% CI: 7.1, 31.4) for women who practiced exclusive lactation compared to those who stopped lactation (Téllez-Rojo et al., 2002).

The mean cortical Pb to current blood Pb ratios for occupationally-exposed subjects are shown in Figure 4-8. Box plots were calculated using data from the following studies: Bergdahl et al., 1998; Brito et al., 2002; Christoffersson et al., 1984; Erfurth et al., 2001; Erkkilä et al., 1992; Fleming et al., 1998; Gerhardsson et al., 1993; Hänninen et al., 1998; Juarez-Perez et al., 2004; Popovic et al., 2005; Roels et al., 1995; Schwartz et al., 2000a,b; Somervaille et al., 1988,

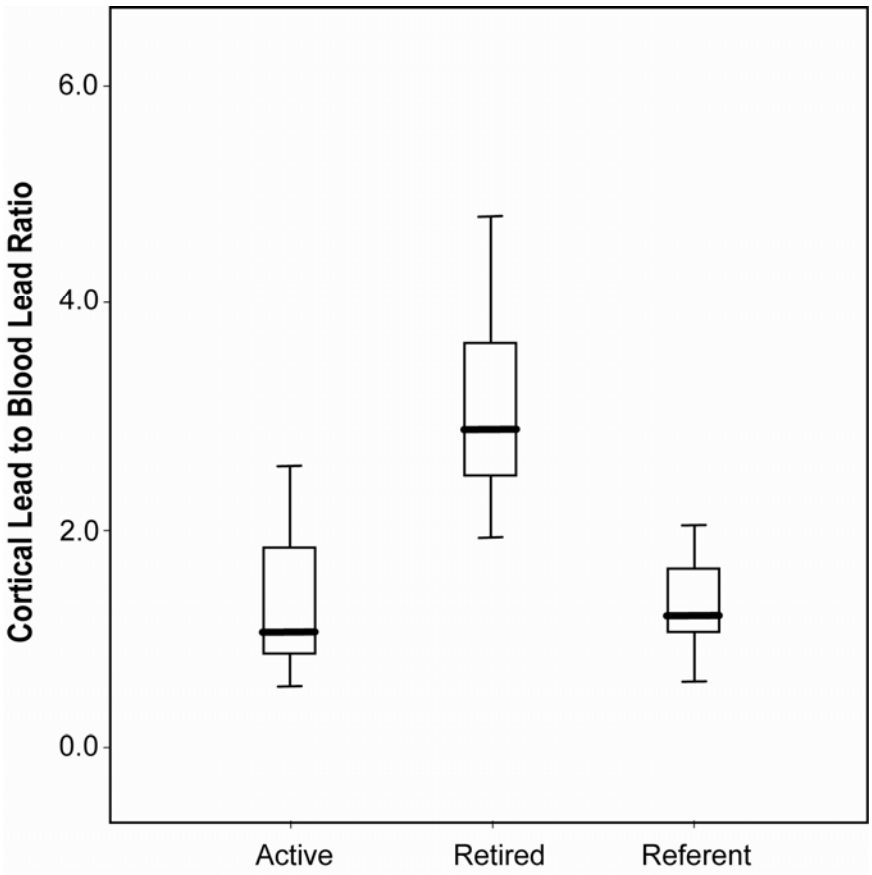

Figure 4-8. Cortical lead to blood lead ratios for occupationally-exposed subjects (both active and retired) and referents. Data compiled from several studies. See text for more details.

1989; Todd et al., 2001. The mean cortical Pb to current blood Pb ratio is about 1.2 (range 0.4-2.6) for active employees (n = 17). For retired employees (n = 7), the mean is 3.2 (range 2.0-5.3), while for environmentally-exposed referent subjects from these industries (n = 7) the mean ratio is about 1.3 (range 1-2.2). The differences in the cortical Pb to blood Pb ratio between active and retired employees and retired employees and referents are significant ($p < 0.01$) but not between active employees and referents. Several investigators have pointed out the weak association between bone Pb and blood Pb in active employees in comparison with the stronger association with retired employees (e.g., Erkkilä et al., 1992; Fleming et al., 1997; Gerhardsson et al., 1993). This is likely because circulatory Pb of active employees reflects

mainly ongoing exposure, whereas that in retired employees is more dependent on Pb released from the skeleton.

The mean tibia Pb to current blood Pb ratios for environmentally-exposed subjects are shown in Figure 4-9. The box plot for pregnancy-related subjects was calculated using data from the following studies: Brown et al., 2000; Chuang et al., 2001; Ettinger et al., 2004; Gomaa et al., 2002; Gonzalez-Cossio et al., 1997; Hernandez-Avila et al., 1996, 1998, 2002, 2003; Hu et al., 1996; Moline et al., 2000; Rothenberg et al., 2000; Sanin et al., 2001; Téllez-Rojo et al., 2002, 2004. The box plot for middle-aged and elderly subjects included the following studies: Berkowitz et al., 2004; Cheng et al., 1998; Garrido-Lattore et al., 2003; Hu et al., 1996, 2001; Korrick et al., 2002; Kosnett et al., 1994; Oliveira et al., 2002; Schafer et al., 2005; Tsaih et al., 2004; Webber et al., 1995. The box plot for the younger subjects (age range 1-30 years) included Farias et al., 1998; Kim et al., 1996; Rosen et al., 1989; Stokes et al., 1998. The mean tibia Pb to blood Pb ratio for pregnancy-related subjects ($n = 21$) is 1.5 (range 1.0-4.2) and is statistically significantly different ($p < 0.001$) from the mean ratio of 3.4 (range 1.6-5.4) for middle-aged to elderly subjects ($n = 27$). Similar relationships are observed for the patella Pb to blood Pb ratios for pregnancy-related subjects and middle-aged to elderly subjects.

In several other studies of environmentally-exposed subjects, there is a stronger relationship between patella Pb and blood Pb than tibia Pb and blood Pb (e.g., Hernandez-Avila et al., 1996; Hu et al., 1996, 1998). Hu et al. (1998) suggest that these relationships indicate that trabecular bone is the predominant bone type providing Pb back into circulation under steady-state and pathologic conditions. The stronger relationships between blood Pb and trabecular Pb compared with cortical bone is probably associated with the larger surface area of trabecular bone allowing for more Pb to bind via ion exchange mechanisms and more rapid turnover making it more sensitive to changing patterns of exposure.

Partitioning of Bone Lead into Plasma

Although most of the Pb in whole blood is associated with erythrocytes (~99%), it has been suggested that the small fraction of Pb in plasma (<0.3%) may be the more biologically labile and toxicologically active fraction of the circulating Pb. Several authors have proposed that Pb released from the skeleton was preferentially partitioned into serum compared with red cells (Cake et al., 1996; Hernandez-Avila et al., 1998; Tsaih et al., 1999) with one explanation

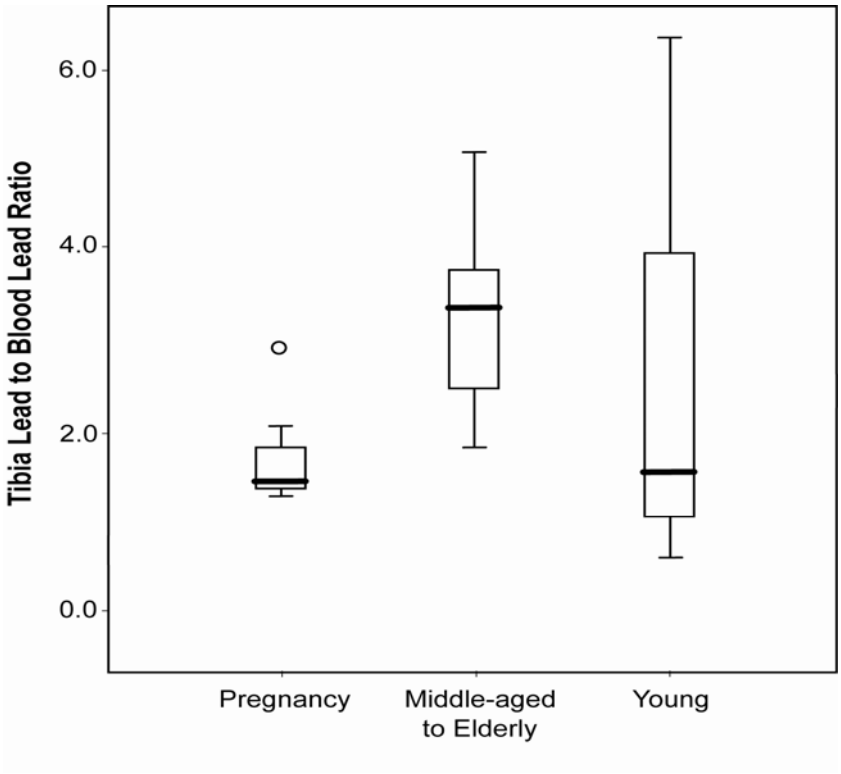

Figure 4-9. Tibia lead to blood lead ratios for environmentally-exposed pregnancy-related subjects, middle-aged to elderly subjects, and younger subjects. Data compiled from several studies. See text for more details.

being that the Pb from endogenous sources was in a different form to that from exogenous sources. In the latter study, Tsaih et al. (1999) suggested that urine was a satisfactory proxy for serum. However, this concept has been withdrawn by its main proponents. In matched blood and urine samples from 13 migrant subjects to Australia who were monitored prior to and during pregnancy, there was no statistically significant difference in the $^{206}Pb/^{204}Pb$ and $^{207}Pb/^{206}Pb$ ratios over pregnancy and the urine results for the postpartum period were in the opposite relationships to those predicted for a preferential partitioning hypothesis (Gulson et al., 2000).

4.3.2.5 Mobilization of Lead From Bone

Although earlier investigators such as Brown and Tompsett (1945), Ahlgren et al. (1976) and Christoffersson et al. (1984) suggested that the skeleton was a potential endogenous source of Pb poisoning, the opposing concept of the skeleton as a "safe" repository for Pb persisted until the mid-1980s and early 1990s. Potential mobilization of Pb from the skeleton could occur at

times of physiological stress associated with enhanced bone remodeling such as during pregnancy and lactation (Hertz-Picciotto et al., 2000; Manton, 1985; Silbergeld, 1991), menopause or in the elderly (Silbergeld, 1991; Silbergeld et al., 1988), extended bed rest (Markowitz and Weinberger, 1990), hyperparathyroidism (Kessler et al., 1999), and weightlessness. The Pb deposited in the bone of adults can serve to maintain blood Pb levels long after exposure has ended (Fleming et al., 1997; Gulson et al., 1995; Inskip et al., 1996; Kehoe, 1987; Manton, 1985; Nilsson et al., 1991; O'Flaherty et al., 1982; Schütz et al., 1987b; Smith et al., 1996).

In the 1986 Lead AQCD, there was a comprehensive summary of chelation therapies and the recognition that there was limited release of Pb from bones. The potential role of bone Pb as an endogenous source of Pb in blood (resulting in elevated levels for former Pb employees) was mentioned, although data to support this hypothesis were limited.

Mobilization of Lead from Bone during Pregnancy and Lactation

Bone Pb studies of pregnant and lactating subjects are summarized in Annex Table AX4-7. Most of the bone XRF studies on pregnancy and lactation have focused on subjects from Mexico City and Latin subjects from Los Angeles, California. Relationships and/or health outcomes from these investigations include: patella bone as a significant contributor to blood Pb (Brown et al., 2000; Hernandez-Avila et al., 1996); a positive association between plasma Pb and bone Pb in the highest bone Pb group of pregnant women (Téllez-Rojo et al., 2004); a positive association of tibia and calcaneus Pb with prenatal Pb concentration, and calcaneus Pb with postnatal Pb (Rothenberg et al., 2000); a positive association of tibia Pb and seasonal variations in blood Pb (Rothenberg et al., 2001); maternal tibia and patella Pb as significant predictors of fetal exposure determined using cord blood (Chuang et al., 2001); a positive association of calcaneus Pb and increased systolic and diastolic blood pressure in the third trimester (Rothenberg et al., 2002); an inverse relationship between maternal tibia and patella Pb, and birth weight (Gonzalez-Cossio et al., 1997; Sanin et al., 2001); an inverse association between tibia Pb and birth length, and patella Pb and head circumference (Hernandez-Avila et al., 2002); an inverse association of maternal patella bone and Mental Development Index (Gomaa et al., 2002); increased bone resorption during lactation (Téllez-

Rojo et al., 2002); increased Pb in breast milk with an increase in patella and tibia Pb (Ettinger et al., 2004).

Lead isotope studies on immigrant women to Australia (Gulson et al., 1997, 1998a) confirmed the earlier work of Manton (1985) of increased blood Pb during pregnancy. Gulson et al. reported that, during pregnancy, blood Pb concentrations in the first immigrant cohort (n = 15) increased by an average of about 20% compared to non-pregnant migrant controls (n = 7). The percentage change in blood Pb concentration was significantly greater during the postpregnancy period than during the second and third trimesters ($p < 0.001$). Skeletal contribution to blood Pb, based on the isotopic composition for the immigrant subjects, increased in an approximately linear manner during pregnancy. The mean increases for each individual during pregnancy varied from 26% to 99%. Skeletal Pb contribution to blood Pb was significantly greater during the postpregnancy period than during the second and third trimesters. The contribution of skeletal Pb to blood Pb during the postpregnancy period remained essentially constant at the increased level of Pb mobilization. In a follow-up study using a different immigrant cohort of 12 women with calcium supplementation at the recommended level of ~1,000 mg/day (National Institutes of Health, 1994), Gulson et al. (2004) found increased mobilization of Pb occurred in the third trimester rather than in the second trimester as observed with first cohort. In addition, the extra flux released from bone during late pregnancy and postpartum varied from 50 to 380 μg (geometric mean 145 μg) compared with 330 μg in the previous cohort.

Also examing blood Pb during pregnancy, Manton et al. (2003) observed that blood Pb concentrations decreased in early pregnancy and rose during late pregnancy. These investigators attributed their results to changes in bone resorption with decoupling of trabecular and cortical bone sites.

Transplacental Transfer of Lead and Transfer through Breast Milk

Transplacental transfer of Pb in humans has been suggested by a number of studies based on cord blood to maternal blood Pb ratios ranging from about 0.6 to 1.0 at the time of delivery. Maternal-to-fetal transfer of Pb appears to be related partly to the mobilization of Pb from the maternal skeleton. Evidence for transfer of maternal bone Pb to the fetus has been provided by stable Pb isotope studies in cynomolgus monkeys (*Macaca fascicularis*). Approximately 7 to

39% of the maternal Pb burden transferred to the fetus appears to derive from the maternal skeleton (Franklin et al., 1997; O'Flaherty et al., 1998). Further evidence for maternal-to-fetal transfer of Pb in humans has been gained from stable Pb isotope measurements. For example, a 0.99 correlation in Pb isotopic ratios for maternal and cord blood (Manton, 1985; Gulson et al., 1998b) and the similarity of isotopic ratios in maternal blood and in blood and urine of newly-born infants provide strong evidence for placental transfer of Pb to the fetus (Gulson et al., 1999b).

Breast milk can also be a pathway of maternal excretion of Pb. However, given the very low Pb concentrations and analytical difficulties arising from high fat contents in breast milk, their analyses require careful attention. Selected studies appear to show a linear relationship between breast milk and maternal whole blood with the percentage of Pb in breast milk compared with whole blood of <3% in subjects for blood Pb concentrations ranging from 2 to 34 µg/dL. Blood Pb concentrations in breastfed newborn infants decreased in spite of the maternal blood Pb concentrations having risen or remained elevated postpartum compared to lower levels during prepregnancy or in the first trimester (Gulson et al., 1999b). Similar trends were noted by Manton et al. (2000). However, in a Mexico City study, an association between patella Pb and blood Pb concentrations was higher for women with partial lactation than for those who stopped lactation, and it was increased among women who breastfed exclusively (Téllez-Rojo et al., 2002). In another Mexico City study, Ettinger et al. (2004) concluded that an interquartile increase in patella Pb was associated with a 14% increase in breast milk Pb, whereas for tibial Pb the increase was ~5%.

In conclusion, there is evidence that maternal-to-fetal transfer of Pb occurs, likely resulting from the mobilization of Pb from the maternal skeleton during pregnancy. Breast-fed infants appear to be at greater risk only if the mother is exposed to high Pb concentrations either from exogenous sources or endogenous sources such as the skeleton.

Mobilization of Lead in Bone during Menopause and in the Elderly

Increases in blood Pb for postmenopausal women have been attributed to release of Pb from the skeleton associated with increased bone remodeling during menopause. Many of the studies have been based on blood Pb concentration. Bone Pb studies of menopausal and middle-aged to elderly subjects are summarized in Annex Table AX4-8.

Overall, the various studies of bone and blood Pb levels, as well as hormone replacement therapy, have provided conflicting outcomes. Hormone replacement therapy alone or combined with calcium supplementation prevents bone resorption and increases the bone mineral density in trabecular and cortical bones of women with or without metabolic bone disease. The effect of hormone replacement therapy may result in a decrease of Pb mobilization from bone along with a reduction in blood Pb levels. Several studies have found that tibia bone Pb levels were higher in women who used hormone replacement therapy (Popovic et al., 2005; Webber et al., 1995). In contrast, other investigators have found no association between bone Pb and use of estrogens (Berkowitz et al., 2004; Korrick et al., 2002). In addition, some studies observed a decrease in blood Pb concentrations associated with hormone replacement therapy (Garrido-Latorre et al., 2003), whereas others observed no association (Webber et al., 1995).

The endogenous release rate of Pb from bone in postmenopausal women was double the rate in premenopausal former smelter employees (Popovic et al., 2005) and environmentally-exposed women from Mexico (Garrido-Latorre et al., 2003). In middle-aged to elderly males from the Normative Aging Study, patella Pb accounted for the dominant portion of variance in blood Pb (Hu et al., 1996).

Effect of Nutritional Status on Mobilization of Lead from Bone

Most studies that investigated the effect of nutritional status on the mobilization of Pb from the skeleton have examined the effects of calcium supplementation. Several studies have suggested that dietary calcium may have a protective role against Pb by decreasing absorption of Pb in the gastrointestinal tract and by decreasing the mobilization of Pb from bone stores to blood, especially during periods of high metabolic activity of the bone such as pregnancy, lactation, and menopause. An inverse association between patella Pb and low calcium intake in postpartum women has been found (Hernandez-Avila et al., 1996). In contrast, Rothenberg et al. (2000) observed that dietary calcium intake had no effect on calcaneus Pb in women monitored during the third trimester and 1 to 2 months postpartum. Likewise, no effect from calcium supplementation on bone Pb was found amongst lactating women from Mexico City (Téllez-Rojo et al., 2002), although in a follow-up study, Hernandez-Avila et al. (2003) reported a 16.4% decrease in blood Pb concentration among women with the highest patella bone Pb levels who were taking supplements. Gulson et al. (2004) observed that calcium supplementation was

found to delay increased mobilization of Pb from bone during pregnancy and halved the flux of Pb release from bone during late pregnancy and postpartum. In another study, women whose daily calcium intake was 850 mg per day showed lower amounts of bone resorption during late pregnancy and postpartum than those whose intake was 560 mg calcium per day (Manton et al., 2003). Téllez-Rojo et al. (2004) observed that plasma Pb levels were inversely related to dietary calcium intake. Results for whole blood Pb were similar but less pronounced.

Some researchers have noted concerns regarding potential Pb toxicity resulting from calcium supplementation. However, Gulson et al. (2001) observed that Pb in calcium or vitamin supplements did not appear to increase blood Pb concentrations. No information was available on the effects of other nutritional supplements (e.g., iron or zinc) on Pb body burden.

4.3.2.6 Summary of Bone Lead as a Biomarker of Lead Body Burden and Exposure

Bone accounts for more than 90% of the total body burden of Pb in adults and 70% in children. In addition, the longer half-life of Pb in bone, which largely depends on the bone type but is generally estimated in terms of years compared to days for blood Pb, allows a more cumulative measure of Pb dose. The more widespread use of in vivo XRF Pb measurements in bone and indirect measurements of bone processes with stable Pb isotopes since the 1986 Lead AQCD have enhanced the use of bone Pb as a biomarker of Pb body burden.

In addition to considering bone Pb as an indicator of cumulative Pb exposure, Pb in the skeleton can also be regarded as a source of Pb. Key studies have examined the contribution of bone Pb to blood Pb; the preferential partitioning of bone Pb into plasma; mobilization of Pb from bones during pregnancy, lactation, and menopause; and the role of nutritional supplementation in bone mobilization.

4.3.3 Lead in Teeth

4.3.3.1 Summary of Key Findings from the 1986 Lead AQCD

The importance of dentine as a potential indicator of Pb exposure was noted in the 1986 Lead AQCD. There was more emphasis and optimism on using dentine to assess Pb exposure in this document as the bone XRF method was in its infancy. The issue of deciduous tooth type was addressed but there was little information on permanent teeth. The portion of the tooth analyzed (i.e., whole tooth or circumpulpal dentine) was also addressed. In the 1990

Supplement, the use of tooth Pb as an exposure metric was described in a number of the longitudinal and cross-sectional studies.

4.3.3.2 Analytical Methods for Measuring Lead in Teeth

Analytical methods for tooth analysis vary from the most widely used AAS, to energy-dispersive XRF, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), and high precision Pb isotopes.

As a standard analytical method has yet to be established for tooth Pb analysis, some of the discrepancies in findings between studies could arise from several factors, including differences in tooth type, part of the tooth analyzed, and tooth location. Any real differences among populations are unlikely to be the result of physiological factors such as blood supply to teeth or mineralization rates. As enamel and dentine in different teeth calcify at overlapping but different times (Orban, 1953), they could retain varying amounts of Pb.

In a systematic evaluation of the magnitude of random errors associated with dentine Pb measurements, Fergusson et al. (1989) measured Pb concentrations in two samples of dentine from 996 New Zealand children. They estimated that 15 to 20% of the variance was unexplained. Tests of differences of means and variances showed no significant differences between the two samples.

Lead measurements in deciduous teeth in individuals from urban and remote environments and from polluted environments are presented in Annex Tables AX4-9 and AX4-10, respectively. Based on the limited number of studies, it would appear that the range in whole deciduous tooth Pb for environmentally exposed subjects is about 1–10 μg/g, but the most likely levels are <5 μg/g and probably even <2 μg/g. Studies of whole deciduous teeth from industrial environments, including those in urban settings, are also commonly much less than 10 μg/g.

The utility of circumpulpal dentine (Shapiro et al., 1973) as the metric of Pb exposure in deciduous teeth has not been enthusiastically received. This is likely due to the separation difficulties, as well as the limited amount of circumpulpal dentine that may be present when the teeth are resorbed, prior to exfoliation.

In another approach to gain more information about exposure during pregnancy and early childhood, the teeth may be sectioned into dominantly enamel or dominantly dentine. These

samples can then be analyzed for Pb isotopic ratios and Pb concentrations (Gulson and Wilson, 1994). Even for children living in Pb mining and smelting communities, Pb levels in the enamel are generally low (<5 μg/g) and are consistent with other studies of whole teeth. However, higher levels are observed in the dentine samples (e.g., 32 μg/g), which likely reflect the early childhood exposure. Permanent teeth tend to have up to three times the level of Pb compared with deciduous teeth, but the number of studies is very limited.

4.3.3.3 Tooth Lead as a Biomarker of Lead Body Burden

Compared with the amount of Pb in the skeleton, tooth Pb is a minor contributor to the body burden of Pb. Most of the tooth Pb information is based on analyses of deciduous teeth. There is still controversy over the amounts of Pb in different whole teeth but it appears that the highest concentrations are in central incisors, with decreasing amounts in lateral incisors, canines, first molars, and second molars. Teeth from the upper jaw tend to have higher Pb concentrations than those from the lower jaw.

As teeth accumulate Pb, tooth Pb levels are generally considered an estimate of cumulative Pb exposure. Rabinowitz et al. (1993) found that tooth Pb was a better measure of exposure than current blood Pb levels; however, it was not a good measure of the child's cumulative exposure from birth to exfoliation due to the mobilization of Pb from dentine.

Teeth are composed of several tissues formed over the years. Therefore, if a child's Pb exposure during the years of tooth formation varied widely, different amounts of Pb would be deposited at different rates (Rabinowitz et al., 1993). This may allow investigators to elucidate the history of Pb exposure in a child.

Gulson and Wilson (1994) advocated the use of sections of enamel and dentine to obtain additional information compared with analysis of the whole tooth (e.g., Fosse et al., 1995; Tvinnereim et al., 1997). For example, deciduous tooth Pb in the enamel provides information about in utero exposure whereas that in dentine from the same tooth provides information about postnatal exposure until the tooth exfoliates at about 6 to 7 years of age.

4.3.3.4 Relationship Between Tooth Lead and Blood Lead

As with bone Pb-blood Pb relationships, there is interest in understanding more about potential relationships between tooth Pb and blood Pb. The tooth Pb-blood Pb relationship is

more complex than the bone Pb-blood Pb relationship because of differences in tooth type, location, and analytical method.

Rabinowitz (1995) used studies which reported values for dentine, whole shed teeth, or crowns, but discarded those measuring circumpulpal dentine because of the higher values in this medium. The mean tooth Pb levels varied from 2.8 to 12.7 µg/g and blood Pb levels from 6.5 to 17 µg/dL. In a plot of blood versus tooth Pb, Rabinowitz found a good fit ($R^2 = 0.97$; $p < 0.0001$) with the relationship:

$$\text{Tooth Lead } (\mu g/g) = \beta \times [\text{Blood Lead } (\mu g/dL)], \text{ where } \beta = 0.49 \text{ (SE 0.04)} \qquad (4\text{-}1)$$

In an earlier Boston study, Rabinowitz et al. (1989) found that the association between tooth and blood Pb increased with age, first achieving statistical significance at 18 months; by 57 months, the correlation coefficient was 0.56. A correlation of 0.47 was found between current blood Pb and incisor Pb concentrations amongst 302 German children (Ewers et al., 1982).

4.3.3.5 Mobilization of Lead from Teeth

Although mobilization of Pb from bone appears well established, this is not the case for Pb in teeth. Conventional wisdom has Pb fixed once it enters the tooth. Although that may be the case for the bulk of enamel, it is not true for the surface of the enamel and dentine.

In evaluating deciduous teeth data, Rabinowitz et al. (1993) suggested that their data were compatible with a model that allows Pb to be slowly removed from dentine. Such a process may be associated with resorption of the root and dentine that precedes exfoliation, which allows reequilibration of dentine Pb with blood Pb.

In children exposed to Pb sources from mining, paint, or petrol in communities such as the Broken Hill Pb mining community, Gulson and Wilson (1994) and Gulson (1996) showed that the source of Pb from the incisal (enamel) sections was different from the source of Pb in the cervical (dentine) sections of deciduous teeth, reflecting the change in Pb from in utero exposure to early childhood. Based on changes in the isotopic composition of enamel and dentine in deciduous teeth sections from the Broken Hill mining community children, Gulson (1996) estimated that Pb is added to dentine at a rate of ~2-3% per year.

Stable Pb isotopes and Pb concentrations were measured in the enamel and dentine of permanent (n = 37) and deciduous teeth (n = 14) from 47 European immigrants to Australia to determine whether Pb exchange occurs in teeth and how it relates to Pb exchange in bone (Gulson et al., 1997). The authors concluded that enamel exhibited no exchange of its European-origin Pb with Pb from the Australian environment, whereas dentine Pb exchanged with Australian Pb to the extent of ~1 ± 0.3% per year.

4.3.3.6 Summary of Tooth Lead as a Biomarker of Lead Body Burden and Exposure

Tooth Pb is a minor contributor to the total body burden of Pb. Moderate-to-high correlations have been observed between tooth Pb levels and blood Pb levels. Differences in tooth type, part of the tooth analyzed, and tooth location may contribute to some of the discrepancies in findings between studies of tooth Pb. As teeth are composed of several tissues formed over the years, if a child's Pb exposure during the years of tooth formation varied widely, different amounts of Pb would be deposited at different rates. Deciduous tooth Pb in the enamel provides information about in utero exposure, whereas that in dentine provides information about postnatal exposure until the tooth exfoliates.

4.3.4 Lead in Urine

4.3.4.1 Summary of Key Findings from the 1986 Lead AQCD

The 1986 Lead AQCD provided an extensive discussion of the physiological basis for "chelatable" urinary Pb. Also discussed was Pb excretion provoked by EDTA, including the pools of Pb in the body that might be mobilized in the EDTA provocation test, and the relationship between the outcome and blood Pb concentration. The 1986 Lead AQCD noted observations that formed the basis for application of the EDTA provocation test for detecting elevated Pb body burden.

4.3.4.2 Analytical Methods for Measuring Lead in Urine

Standard methods that have been reported for urine Pb analysis are summarized in Annex Table AX4-1 and are, in general, the same as those analyses noted for determination of Pb in blood. Reported detection limits are ~50 µg/L for AAS, 5–10 µg/L for ICP AES, and 4 µg/L for ASV for urine Pb analyses. Sample preparation usually consists of wet ashing; however,

chelation and solvent extraction has also been reported (National Institute for Occupational Safety and Health, 1994, 1977a).

4.3.4.3 Levels of Lead in Urine

A summary of selected measurements of urine Pb levels in humans can be found in Annex Table AX4-11. Urine Pb concentrations in the U.S. general population have been monitored in NHANES. Data from the most recent survey (NHANES IV, Centers for Disease Control, 2005) for subjects ≥6 years of age are shown in Table 4-4. The geometric mean for the entire sample (n = 2,689) was 0.64 µg/g creatinine (95% CI: 0.60, 0.68). The geometric means for males (n = 1,334) and females (n = 1,335) were 0.64 µg/g creatinine (95% CI: 0.61, 0.67) and 0.64 µg/g creatinine (95% CI: 0.59, 0.69), respectively. These values correspond to an excretion rate of ~1-1.3 µg Pb/day for an adult, assuming a daily creatinine excretion rate of ~1.5 g/day in adult females, a body weight of 70 kg for males and 58 kg for females, and a lean body mass fraction of 0.88 for males and 0.85 for females (Forbes and Bruining, 1976; International Commission on Radiological Protection, 1975).

Table 4-4. Urine Lead Concentrations in U.S. by Age, NHANES IV (1999–2002)

Age	6–11 years		12–19 years		≥20 years	
Survey Period	1999–2000	2001–2002	1999–2000	2001–2002	1999–2000	2001–2002
N	340	368	719	762	1406	1559
Urine Lead[a]	1.17 (0.98, 1.41)	0.92 (0.84, 1.00)	0.50 (0.46, 0.54)	0.40 (0.38, 0.43)	0.72 (0.68, 0.76)	0.66 (0.62, 0.70)

[a]Urine Pb concentrations presented are geometric means (95% CI) of µg-Pb/g-creatinine.

Geometric mean urinary Pb excretion rates of 7-10 µg/g creatinine (maximum 43) have been reported in groups of children living in areas impacted by Pb smelting operations (Brockhaus et al., 1988). Daily urinary Pb excretion can exceed 200 µg/day in association with occupational exposures (Biagini et al., 1977; Cramer et al., 1974; Lilis et al., 1968; Lin et al., 2001; Wedeen et al., 1975).

4.3.4.4 Urine Lead as a Biomarker of Lead Body Burden

Urine is a major route of excretion of absorbed Pb (Chamberlain et al., 1978; Griffin et al., 1975; Kehoe, 1987; Rabinowitz et al., 1976). The kinetics of urinary excretion following a single dose of Pb is similar to that of blood (Chamberlain et al., 1978), likely due to the fact that Pb in urine derives largely from Pb in blood plasma. Evidence for this is the observation that urinary Pb excretion is strongly correlated with the rate of glomerular filtration of Pb (i.e., glomerular filtration rate × plasma Pb concentration; Araki et al., 1986). Estimates of urinary clearance of Pb from serum (or plasma) range from 13-22 L/day, with a mean of 18 L/day (Araki et al., 1986; Chamberlain et al., 1978; Manton and Cook, 1984; Manton and Malloy, 1983). Estimates of blood-to-urine clearance, on the other hand, range from 0.03-0.3 L/day with a mean of 0.12 L/day (Araki et al., 1990; Berger et al., 1990; Chamberlain et al., 1978; Gulson et al., 2000; Koster et al., 1989; Manton and Malloy, 1983; Rabinowitz et al., 1973, 1976; Ryu et al., 1983; see Diamond, 1992 for an analysis of these data), consistent with a plasma to blood concentration ratio of ~0.005–0.01 L/day (U.S. Environmental Protection Agency, 2003b). Based on the above, urinary excretion of Pb can be expected to reflect the concentration of Pb in plasma and variables that affect delivery of Pb from plasma to urine (e.g., glomerular filtration and other transfer processes in the kidney).

Plasma Pb makes a small contribution (<1%) to the blood Pb concentration and a negligible contribution to total Pb body burden. Furthermore, the kinetics of elimination of Pb from plasma is fast, relative to Pb in bone, where most of the Pb burden resides. Therefore, the basic concepts described for blood as a biomarker for Pb body burden also apply to urine. A single urine Pb measurement, or a series of measurements taken over short-time span, is likely a relatively poor index of Pb body burden (Figure 4-10). On the other hand, long-term average measurements of urinary excretion can be expected to be a better index of body burden. In the hypothetical simulation shown in Figure 4-10, both the long-term average urinary Pb excretion rate and the body burden have approximately doubled.

The above considerations do not exclude the potential utility of urine Pb as a dose metric in epidemiological studies. Some effect outcomes may be more strongly associated with plasma concentrations of Pb (e.g., ferrochelatase inhibition) than Pb body burden. Given the technical difficulties in accurately measuring the concentrations of Pb in plasma, especially at low blood

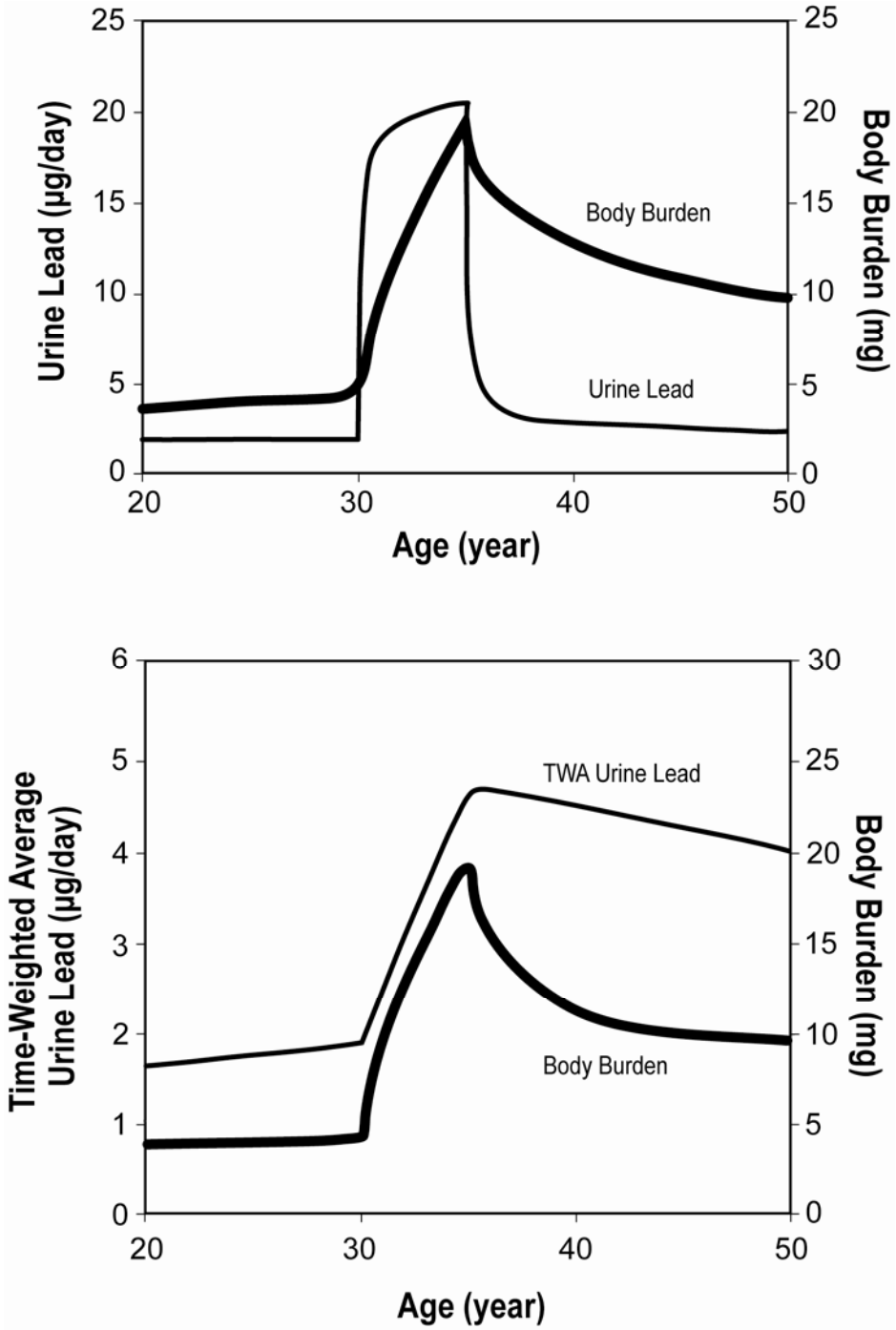

Figure 4-10. Simulation of relationship between urinary lead excretion and body burden in adults. An abrupt change in lead uptake gives rise to a relatively rapid change in urinary excretion of lead, to a new quasi-steady state, and a relatively small change in body burden (upper panel). The long-term average urinary lead excretion more closely tracks the pattern of change in body burden (lower panel). Simulation based on Leggett (1993) lead biokinetics model.

Pb levels (e.g., <10 µg/dL), measurements of urinary Pb may serve as a more feasible surrogate for measurements of plasma Pb concentration.

4.3.4.5 Relationship Between Lead in Blood and Urine

Assuming first-order kinetics, a plasma-to-urine clearance (UCl_P) of 13-22 L/day corresponds to half-time for transfer of Pb from plasma to urine of 0.1-0.16 day for a 70 kg adult who has a plasma volume (VP) of ~3 L:

$$t_{1/2} = \frac{\text{In}(2) \cdot V_P}{UCl_P} \tag{4-2}$$

This translates to a very rapid steady-state, much faster than observed for blood Pb after a change in exposure level. The kinetics of change in urinary Pb excretion in response to a change in exposure, therefore, will be determined by variables that affect the plasma Pb level, including partitioning of Pb into erythrocytes and exchanges with Pb in soft tissues and mobile pools within bone (e.g., bone surface). Here again, the basic concepts that apply to blood Pb as a biomarker of exposure also apply to urine Pb. Urinary Pb excretion reflects, mainly, the exposure history of the previous few months; thus, a single urinary Pb measurement cannot distinguish between a long-term low level of exposure or a higher acute exposure. The relationship between urinary Pb concentration and Pb uptake is thought to be linear, unlike that for blood Pb concentration, although there are no direct empirical tests of this assumption in humans. This assumption predicts a linear relationship between Pb intake (at constant absorption fraction) and urinary Pb excretion rate. Figure 4-11 presents a simulated relationship between Pb intake and urinary Pb excretion in adults and children using both the Leggett (1993) model and O'Flaherty (1993, 1995) model. The major difference between the Leggett model and the O'Flaherty model is in the assignment of the time dependence of bone Pb residence. The Leggett model assumes a slow accumulation of a nonexchangable Pb pool, whereas the O'Flaherty model assumes a gradual distancing of Pb from bone surfaces by diffusion throughout the bone volume (O'Flaherty, 1998).

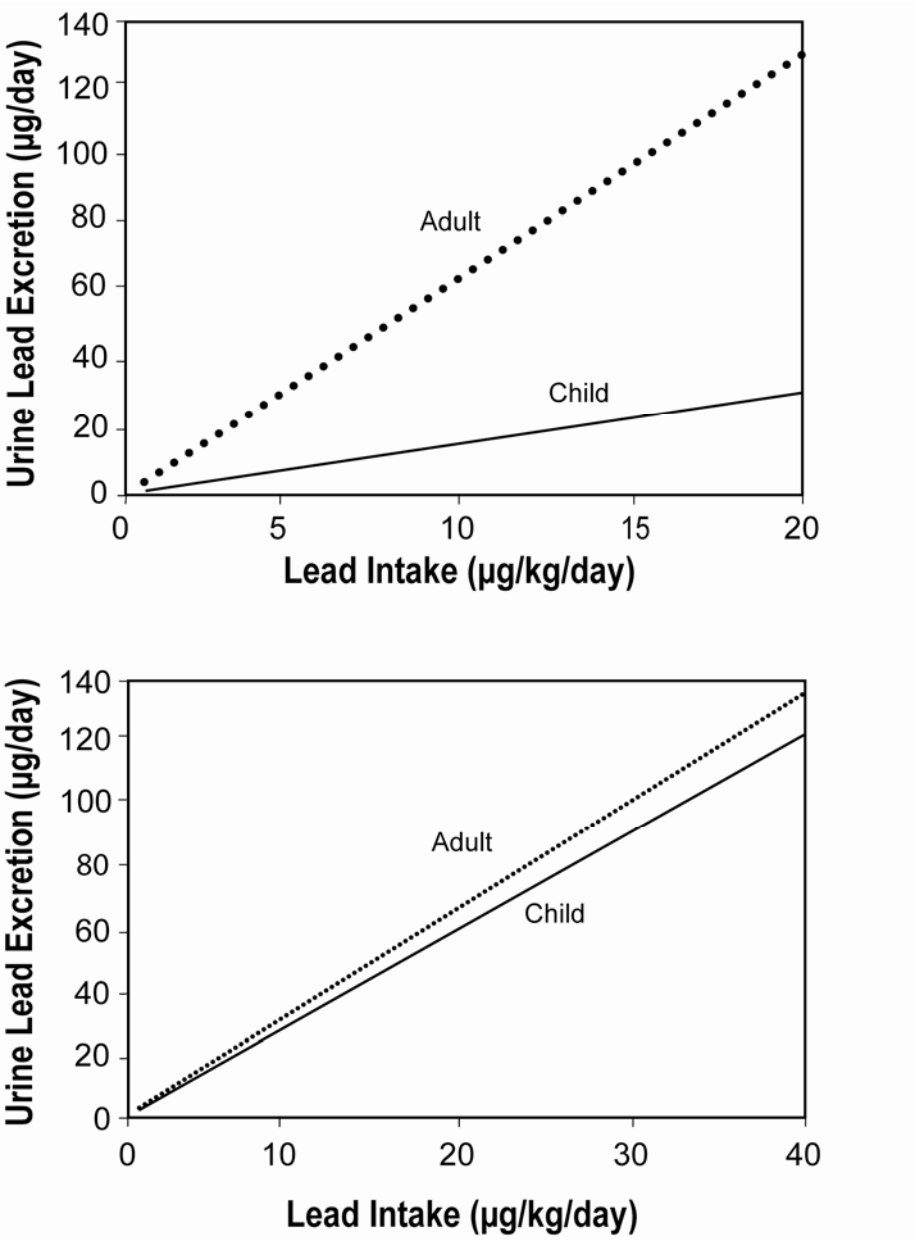

Figure 4-11. Simulation of relationship between lead intake and urinary lead excretion in adults and children. Predictions are for a 2-year-old child and 30-year-old adult, for a constant lead intake (µg/kg/day). The relationship is linear, for intake and plasma lead concentration (not shown). Predictions are based on Leggett (1993, upper panel) and O'Flaherty (1993, 1995, lower panel).

It is important to emphasize that the above concepts apply to urinary Pb excretion rate, not to urinary Pb concentration. The concentration of Pb in urine (U_{Pb}) is a function of the urinary Pb excretion (UE_{Pb}) and the urine flow rate (UFR, L/day):

$$UE_{Pb} = U_{Pb} \cdot UFR \tag{4-3}$$

Urine flow rate can vary by a factor or more than 10, depending on the state of hydration and other factors that affect glomerular filtration rate and renal tubular reabsorption of the glomerular filtrate. All of these factors can be affected by Pb exposure at levels that produce nephrotoxicity (i.e., decreased glomerular filtration rate, impaired renal tubular transport function; see Section 6.4 for discussion of effects of Pb on the renal system). Therefore, urine Pb concentration measurements provide little reliable information about exposure (or Pb body burden), unless they can be adjusted to account for unmeasured variability in urine flow rate (Araki et al., 1990).

A determination of urinary Pb excretion rate requires measurement of two variables, urine Pb concentration, and urine flow rate; the latter requires collection of a timed urine sample, which is often problematic in epidemiologic studies. Collection of un-timed ("spot") urine samples, a common alternative to timed samples, requires adjustment of the Pb measurement in urine to account for variation in urine flow (Diamond, 1988). Several approaches to this adjustment have been explored, including adjusting the measured urine Pb concentration by the urine creatinine concentration, urine osmolality, or specific gravity (Araki et al., 1990).

The measurement of Pb excreted in urine following an injection (intravenous or intramuscular) of the chelating agent calcium disodium EDTA (EDTA provocation) has been used to detect elevated body burden of Pb in adults (Biagini et al., 1977; Lilis et al., 1968; Wedeen, 1992; Wedeen et al., 1975) and children (Chisolm et al., 1976; Markowitz and Rosen, 1981). EDTA-provoked urinary Pb excretion has been shown to correlate with tibia bone Pb measurements (Wedeen, 1992). Given difficulties associated with parenteral administration of EDTA, XRF measurements of bone Pb, offer a more feasible alternative to the EDTA provocation test for assessment of bone Pb stores in epidemiologic studies. More recently, DMSA (DMSA-provocation) has been used as an orally-effective alternative to EDTA and has been applied to epidemiologic studies as dose metric for Pb body burden (e.g., Lee et al., 2001; Schwartz et al., 2001, 2000a,b).

4.3.4.6 Summary of Urine Lead as a Biomarker of Lead Body Burden and Exposure

Similar to blood Pb concentration measurements, urinary Pb excretion measured in an individual at a single point in time will reflect the recent exposure history of the individual and physiological variables that determine the plasma Pb concentration time profile. As a result, measurement of urinary Pb may serve as a more feasible surrogate for plasma Pb concentration, and may be useful for exploring dose-response relationships for effect outcomes that may be more strongly associated with plasma Pb concentration than Pb body burden. Longitudinal measurements of urinary Pb excretion can be expected to provide a more reliable measure of exposure history of an individual and will more closely parallel body burden than will single measurements; however, the degree to which this will apply will depend on the sampling frequency with respect to the exposure temporal pattern.

Although, in general, higher urinary Pb excretion can be interpreted as indicating higher exposures (or Pb uptakes), it does not necessarily predict appreciably higher body burdens. Similar urinary Pb excretion rates in two individuals (or populations) do not necessarily translate to similar body burdens or similar exposure histories.

Measurement of the urinary Pb excretion rate requires either a timed urine sample, or an approach to adjusting measured urinary Pb concentrations for variability in urine flow rate, which by itself may be affected by Pb exposure (i.e., Pb-induced nephrotoxicity). Both approaches, timed urine samples or adjustment of concentration, introduce complications into the assessment and uncertainties into the interpretation of urinary Pb measurements as biomarkers of Pb body burden or exposure. The EDTA-provocation test provides a more reliable indicator of elevated Pb body burden than do measurements of basal Pb excretion; however, it is not feasible to apply this test for epidemiologic investigations. The DMSA-provocation test may provide a more feasible alternative.

4.3.5 Lead in Hair

4.3.5.1 Summary of Key Findings from the 1986 Lead AQCD

The 1986 Lead AQCD did not discuss applications of hair Pb measurements for assessing Pb body burden or exposure.

4.3.5.2 Analytical Methods for Measuring Lead in Hair

Methods used for hair Pb analysis are summarized in Annex Table AX4-1. Wilhelm et al. (1989) reported a detection limit of 0.16 µg/g for GFAAS; use of GFAAS for hair Pb measurements has been reported elsewhere (Annesi-Maesano et al., 2003). Gerhardsson et al. (1995a) reported a detection limit of 0.5 µg/g for XRF of the hair shaft; but Campbell and Toribara (2001) found XRF to be unreliable for hair root Pb determinations. Use of other methods has been reported, including ICP (Tuthill, 1996), ET/AAS (Drasch et al., 1997), and AAS (Sharma and Reutergardh, 2000; Esteban et al., 1999).

4.3.5.3 Levels of Lead in Hair

A summary of selected measurements of hair Pb levels in humans can be found in Annex Table AX4-12. Reported hair Pb levels vary considerably. Esteban et al. (1999) reported a geometric mean level of 5.4 ng/g (range 1-39) for a sample of 189 children (aged 1.9 to 10.6 years) residing in Russian towns impacted by smelter and battery plant operations. By contrast, Tuthill (1996) reported much higher levels in a sample of Boston, MA children (aged 6.5 to 7.5 years, n = 277). Approximately 41% had levels that ranged from 1 to 1.9 µg/g. DiPietro et al. (1989) reported a geometric mean hair Pb level of 2.42 µg/g (10–90th percentile range <1.0-10.8) in a general population sample of U.S. adults (aged 20 to 73 years, n = 270). In a post-mortem sample of the general population from Germany (aged 16 to 93 years, n = 150), the median hair Pb level was 0.76 µg/g (range 0.026-20.6) (Drasch et al., 1997). Also, Gerhardsson et al. (1995a) reported median values for postmortem samples of 8.0 µg/g (range 1.5-29,000) in active workers (n = 6), 2.6 µg/g (range 0.6-9.3) in retired workers (n = 23), and 2.1 µg/g (range 0.3-96) in a reference group (n = 10).

4.3.5.4 Hair Lead as a Biomarker of Lead Body Burden

Lead is incorporated into human hair and hair roots (Bos et al., 1985; Rabinowitz et al., 1976) and has been explored as a possibly noninvasive approach for estimating Pb body burden (Gerhardsson et al., 1995a; Wilhelm et al., 1989, 2002). Hair Pb measurements are subject to error from contamination of the surface with environmental Pb and contaminants in artificial hair treatments (i.e., dyeing, bleaching, permanents) and are a relatively poor predictor of blood Pb concentrations, particularly at low levels (<10 to 12 µg/dL) (Campbell and Toribara, 2001;

Drasch et al., 1997; Esteban et al., 1999). Studies evaluating quantitative relationships between hair Pb and Pb body burden have not been reported. Nevertheless, hair Pb levels have been used as a dose metric in some epidemiologic studies (e.g., Annesi-Maesano et al., 2003; Esteban et al., 1999; Gerhardsson et al., 1995a; Powell et al., 1995; Sharma and Reutergardh, 2000; Tuthill, 1996).

4.3.5.5 Hair Lead as a Biomarker of Lead Exposure

Rabinowitz et al. (1976) measured hair Pb levels in two adult males who received a stable Pb isotope supplement to their dietary intake for 124–185 days. Approximately 1% of the daily Pb intake was recovered in hair. Temporal relationships between exposure levels and kinetics and hair Pb levels, and kinetics of deposition and retention of Pb in hair have not been evaluated. Higher hair Pb levels were observed in Pb workers than in reference subjects with lower blood Pb levels (Mortada et al., 2001).

4.3.5.6 Summary of Hair Lead as a Biomarker of Lead Body Burden and Exposure

Although hair Pb measurements have been used in some epidemiologic studies, an empirical basis for interpreting hair Pb measurements in terms of body burden or exposure has not been firmly established. Hair Pb measurements are subject to error from contamination of the surface with environmental Pb and contaminants in artificial hair treatments (i.e., dyeing, bleaching, permanents) and, as such, are relatively poor predictor of blood Pb concentration, particularly at low levels (<10 to 12 μg/dL).

4.4 MODELING LEAD EXPOSURE AND TISSUE DISTRIBUTION OF LEAD

4.4.1 Introduction

Models are essential for quantifying human health risks that derive from exposures to Pb. Models come in various forms. Multivariate regression models, commonly used in epidemiology, provide estimates of the contribution of variance in the internal dose metric to various determinants or control variables (e.g., surface dust Pb concentration, air Pb concentration). Structural equation modeling links several regression models together to

estimate the influence of determinants on the internal dose metric. Regression models can provide estimates of the rate of change of blood or bone Pb concentration in response to an incremental change in exposure level (i.e., slope factor). A strength of regression models is that they are empirically verified within the domain of observation and have quantitative estimates of uncertainty imbedded in the model structure. However, regression models are based on (and require) paired predictor-outcome data, and, therefore, the resulting predictions are confined to the domain of observations. Regression models also frequently exclude numerous parameters that are known to influence human Pb exposures (e.g., soil and dust ingestion rates) and the relationship between human exposure and tissue Pb levels, parameters which are expected to vary spatially and temporally. Thus, extrapolation of regression models to other spatial or temporal contexts, which is often necessary for regulatory applications of the models, can be problematic.

An alternative to regression models are mechanistic models, which attempt to specify all parameters needed to describe the mechanisms (or processes) of transfer of Pb from the environment to human tissues. Such mechanistic models more complex than regression models; this added complexity introduces challenges in terms of their mathematical solution and empirical verification. However, by incorporating parameters that can be expected to vary spatially or temporally, or across individuals or populations, mechanistic models can be extrapolated to a wide range of exposure scenarios, including those that may be outside of the domain of paired predictor-outcome data used to develop the model. Exposure-intake models, a type of mechanistic models, are highly simplified mathematical representations of relationships between levels of Pb in environmental media and human Pb intakes (e.g., µg Pb ingested per day). These models include parameters representing processes of Pb transfer between environmental media (e.g., air to surface dust) and to humans, including rates of human contact with the media and intakes of the media (e.g., g soil ingested per day). Intake-biokinetic models provide the analogous mathematical representation of relationships between Pb intakes and Pb levels in body tissues (e.g., blood Pb concentration); and they include parameters that represent processes of Pb transfer (a) from portals of entry into the body and (b) from blood to tissues and excreta. Linked together, exposure-intake and intake-biokinetics models (i.e., integrated exposure-intake-biokinetics models) provide an approach for predicting blood Pb concentrations (or Pb concentrations in other tissues) that corresponds to a specified exposure (medium, level,

and duration). Detailed information on exposure and internal dose can be obtained from controlled experiments, but almost never from epidemiological observations or from public health monitoring programs. Exposure intake-biokinetics models can provide these predictions in the absence of complete information on the exposure history and blood Pb concentrations for an individual (or population) of interest. Therefore, these models are critical to applying epidemiologically-based information on blood Pb-response relationships to the quantification and characterization of human health risk. They are also critical for assessing the potential impacts of public health programs directed at mitigation of Pb exposure or of remediation of contaminated sites.

Models (both regression models and mechanistic models) also have several other important features that are useful for risk assessment and for improving our basic understanding of Pb exposures and biokinetics. They organize complex information on Pb exposure and biokinetics into a form that provides predictions that can be quantitatively compared to observations. By analyzing the relationships between model assumptions and predictions (i.e., sensitivity analysis) and by comparing predictions to observations (i.e., model evaluation), such models can contribute to the identification of important gaps in our understanding of Pb exposure, biokinetics, and risk. Thus, these models provide a consistent method for making, evaluating and improving predictions that support risk assessment and risk management decisions.

Modeling of human Pb exposures and biokinetics has advanced considerably during the past several decades. Among the most important new advances are development, evaluation, and extensive application of the Integrated Exposure Uptake Biokinetic (IEUBK) Model for Lead in Children (U.S. Environmental Protection Agency, 1994a) and the development of models that simulate Pb biokinetics in humans from birth through adulthood (Leggett, 1993; O'Flaherty 1993, 1995). While these developments represent important conceptual advances, several challenges remain for further advancements in modeling and applications to risk assessment. The greatest challenge derives from the complexity of the models. Human exposure-biokinetics models include large numbers of parameters, which are required to describe the many processes that contribute to Pb intake, absorption, distribution, and excretion. The large number of parameters complicates the assessment of confidence in parameter values, many of which cannot be directly measured. Statistical procedures can be used to evaluate the degree to which model

outputs conform to "real-world" observations and values of influential parameters can be statistically estimated to achieve good agreement with observations. Still, large uncertainty can be expected to remain about many, or even most, parameters in complex exposure-biokinetic models such as those described below. Such uncertainties need to be identified and their impacts on model predictions quantified (i.e., through use of sensitivity analysis, probabilistic methods).

Given the difficulty in quantitatively assessing uncertainty in values of all of the individual parameters in an exposure-biokinetics model, assurance that the model accurately represents the real-world in all aspects is virtually impossible. As consequence of this, Oreskes (1998) noted, *"…the goals of scientists working in a regulatory context should be not validation but evaluation, and where necessary, modification and even rejection. Evaluation implies an assessment in which both positive and negative results are possible, and where the grounds on which a model is declared, good enough are clearly articulated."* In this context, evaluation of confidence in a given exposure-intake or intake-biokinetics model rests largely on assessment of the degree to which model predictions, based on model inputs appropriate for a situation, conform to observations and/or expectations; and, most importantly, the degree to which this conformity does or does not satisfy requirements of model application to a specific context. Because of limitations in observations of predicted outcomes, it may be possible to evaluate confidence in some uses of a model, but not others. Similarly, it is possible for confidence in a model to be judged acceptable for a given use, but not for others. The concept of *validation* of highly complex mechanistic models, outside of the context of a specific use of the model, has little meaning. In this chapter, discussions of specific models include references to sources of information on quantitative assessments of uncertainty of specific model applications. These assessments have been limited to assessments of model performance for prediction of impacts of surface dust Pb exposures on blood Pb concentrations in children.

In the ensuing discussion of specific models, reported efforts to evaluate the models are noted. In most cases, however, the relevance of these evaluations to the assessment of confidence in a specific use of that model (e.g., predicting average blood Pb concentrations in children who live in areas that have certain cross-sectionally measured environmental Pb levels) cannot be ascertained from the reported literature. Nevertheless, as a framework for qualitatively comparing the various evaluative procedures that have been applied, the following general classification of model evaluations has been adopted:

- Sensitivity analyses have been conducted and most influential parameters identified and uncertainty characterized.
- Model predictions have been compared qualitatively to observations.
- Predictions have been compared quantitatively to observations (i.e., a statistical model has been applied for estimation of "goodness of fit" and uncertainty).
- Confidence in model predictions for specific uses has been quantitatively evaluated.
- Accuracy of model implementation code has been verified.

Descriptions of the individual models are intended to provide only brief snapshots of key features of each model, with particular attention to conceptual features that are unique to each model. Key references are cited in which more complete specifications of model parameters can be found.

4.4.2 Slope Factor Models

Empirically-based relationships between blood Pb concentrations and Pb intakes and/or Pb levels in environmental media have provided the basis for what has become known as slope factor models. Slope factor models are highly simplified representations of empirically-based regression models in which the slope parameter represents the change in blood Pb concentration projected to occur in association with a change in Pb intake or uptake. The slope parameter is factored by exposure parameters (e.g., exposure concentrations, environmental media intake rates) that relate exposure to blood Pb concentration (Maddaloni et al., 2005; U.S. Environmental Protection Agency, 2003c; Abadin and Wheeler, 1997; Stern, 1996; Bowers et al., 1994; Stern, 1994; Carlisle and Wade, 1992). In slope factor models, Pb biokinetics are represented as a linear function between the blood Pb concentration and either Pb uptake (uptake slope factor, USF) or Pb intake (intake slope factor, ISF). The models take the general mathematical forms:

$$PbB = E \cdot ISF \tag{4-4}$$

$$PbB = E \cdot AF \cdot USF \tag{4-5}$$

where PbB is the blood Pb concentration, E is an expression for exposure (e.g., soil intake × soil Pb concentration) and AF is the absorption fraction for Pb in the specific exposure medium of

interest. Intake slope factors are based on ingested rather than absorbed Pb and, therefore, integrate both absorption and biokinetics into a single slope factor, whereas models that utilize an uptake slope factor include a separate absorption parameter. In contrast to mechanistic models, slope factor models predict quasi-steady state blood Pb concentrations that correspond to time-averaged daily Pb intakes (or uptakes) that occur over sufficiently long periods to produce a quasi-steady state (i.e., >75 days, ~3 times the $t_{1/2}$ for elimination of Pb in blood).

The U.S. EPA Adult Lead Methodology (ALM) is a example of a slope factor model that has had extensive regulatory use in the EPA Superfund program (Maddaloni et al., 2005); it is the model recommended by EPA for assessing health risks to adults associated with non-residential exposures to Pb in contaminated soils (U.S. Environmental Protection Agency, 1996a). The model was developed to predict maternal and fetal blood Pb concentrations that might occur in relation to maternal exposures to soils contaminated. The model is implemented with the following algorithms (see Table 4-5 for explanation of parameters):

$$PbB_{adult,central} = PbB_{adult,0} + \frac{PbS \cdot BKSF \cdot IR_S \cdot AF_S \cdot EF_S}{AT} \tag{4-6}$$

$$PbB_{fetal,95thpercentile} = PbB_{adultrcentral} \cdot GSD_i^{1.645} \cdot R \tag{4-7}$$

$$\mathrm{PbB_{adult,central,goal}} = \frac{\mathrm{PbB_{fetal,95,goal}}}{\mathrm{GSD_{i,adult}^{1.645} \cdot R_{fetal,maternal}}} \tag{4-8}$$

$$\mathrm{RBC_S} = \frac{(\mathrm{PbB_{adult,central,goal}} - \mathrm{PbB_{adult,0}}) \cdot \mathrm{AT}}{\mathrm{BKSF \cdot IR_S \cdot AF_S \cdot EF_S}} \tag{4-9}$$

The ALM partitions the contributions of Pb exposure on blood Pb concentration of adults ($PbB_{adult,central}$) into two sources: exposure to non-residential site soil, including outdoor soil and indoor soil-derived dust and off-site (*e.g.,* residential) exposures to all other media; the latter contribute to a baseline blood Pb concentration ($PbB_{adult,0}$). If the risk-based goal is to ensure that there is no more than a 5% probability that a fetus will have a blood Pb concentration of 10 µg/dL, then a risk-based goal for the blood Pb concentration in the adult ($PbB_{adult,central,goal}$) is

Table 4-5. Recommended Parameter Values for the Adult Lead Methodology (ALM) and Corresponding Risk-based Soil Lead Concentrations (RBC_S)[a]

Parameter	Value	Unit	Comment
$PbB_{fetal,95,\ goal}$	10	µg/dL	Goal for the 95th percentile blood Pb concentration (µg/dL) among potential fetuses of women exposed to the RBC_{PbS}.
$GSD_{i,adult}^{1.645}$	2.1–2.3[a]	—	Inter-individual geometric standard deviation of PbBs among women of child-bearing age that have exposures to similar on-site Pb concentrations.
$R_{fetal/maternal}$	0.9	—	Proportionality between fetal PbB at birth and maternal PbB.
$PbB_{adult,central}$	Calculated value	µg/dL	Typical PbB concentration (µg/dL) in women of child-bearing age at the site in the absence of exposures to the site that is being assessed.
$PbB_{adult,0}$	1.5–1.7[b]	µg/dL	Baseline PbB, the typical PbB in women of child-bearing age at the site in the absence of exposures to the site.
Pb_S	User Specified value	ppm	Concentration of Pb in soil (µg/g)
BKSF	0.4	µg/dL per µg/day	BKSF = biokinetic slope factor; ratio of (quasi-steady state) increase in typical adult PbB concentration to average daily Pb uptake (µg/dL PbB increase per µg/day Pb uptake)
IR_S	0.05	g/day	Combined intake rate of soil, including both outdoor soil and indoor soil-derived dust.
AF_S	0.12	—	Absolute gastrointestinal absorption fraction for ingested Pb in soil and Pb in dust derived from soil.
EF_S	219	days/year	Exposure frequency for contact with assessed soils and/or dust derived in part from these soils (days of exposure during the averaging period).
AT	365	days/year	Averaging time, the total period during which soil contact may occur.
RBC_S	Calculated value	ppm	Risk-based soil Pb concentration (RBC) that would be estimated to result in a specified central tendency PbB concentrations in adults (i.e., women of child-bearing age) at the site ($PbB_{adult,central,goal}$) and corresponding 95th percentile fetal PbB concentration ($PbB_{fetal,0.95,goal}$).

[a]Based on U.S. EPA (1996a).

[b]$GSD_{i,adult}^{1.645}$ and $PbB_{adult,0}$ are for women of ages 17–45 years as reported in the combined NHANES III Phases 1 and 2 (U.S. EPA, 2002).

given by Equation (4-8), and the corresponding risk-based site soil Pb concentration (RBC_S) is given by Equation (4-9). Figure 4-12 shows the predicted relationship between soil Pb concentration and 95th percentile fetal blood Pb concentration. The predictions correspond to values GSDi=2.1 and PbB_0=1.5 ug/dL, the central estimates of these parameters for the U.S. adult population (age range: 17-45 years, U.S. EPA 2002). The soil Pb concentration that corresponds to 95th percentile=10 µg/dL is ~1250 ppm.

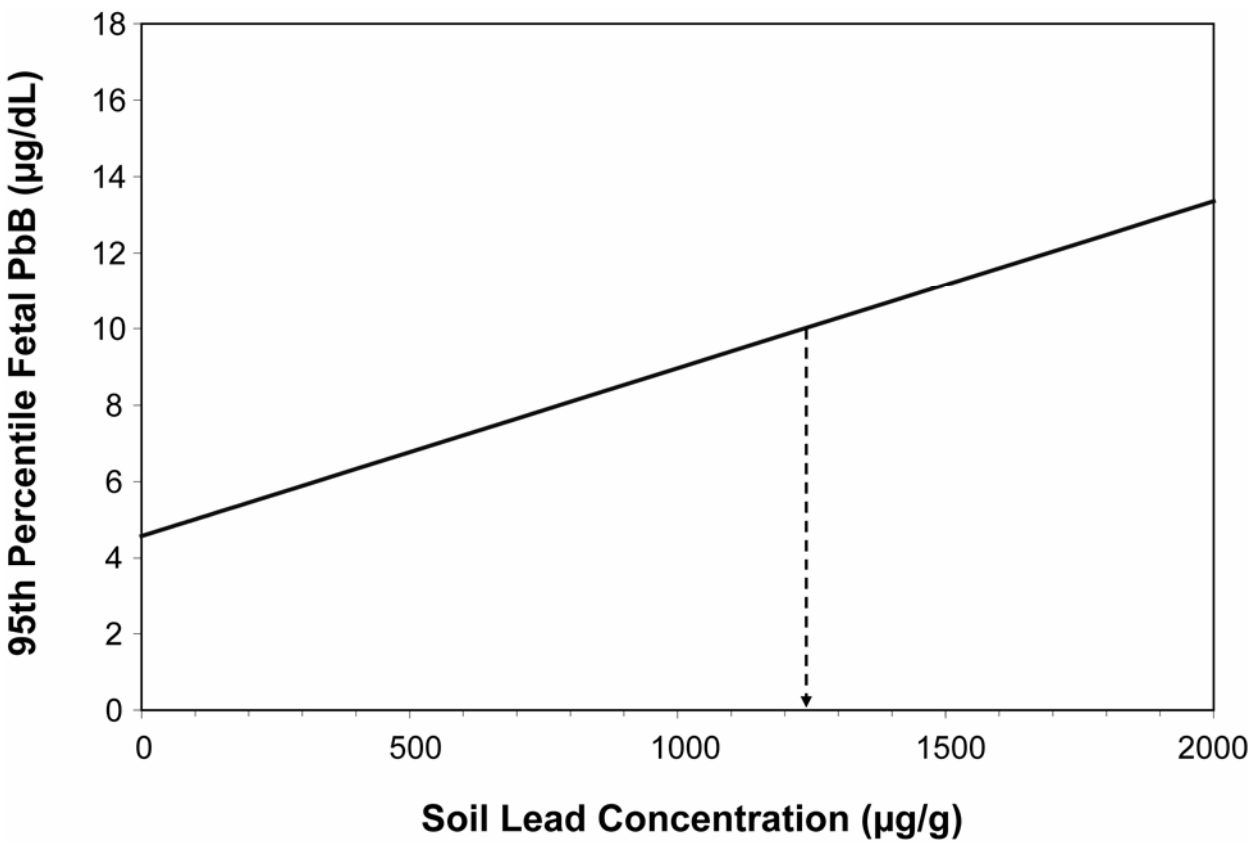

Figure 4-12. Adult lead model (ALM) predictions of the relationship between soil lead concentration and 95th percentile fetal blood lead concentration. The line represents predictions for assumed values of GSD_i=2.1 and PbB_0=1.5 ug/dL. All other parameter values are from Table 4-5. The soil lead concentration that corresponds to 95th percentile=10 ug/dL is ~1250 ppm.

4.4.3 Empirical Models of Lead Exposure-Blood Lead Relationships

The 1986 Lead AQCD described epidemiological studies that explored models of relationships between Pb exposures and blood Pb concentrations in children. A more recent summary of this literature can be found in Abadin et al. (1997). Key studies reported since the completion of the 1986 Lead AQCD are summarized here. Although varying widely in exposure scenarios, blood Pb concentration ranges, and modeling approaches, most studies have found significant associations between surface dust Pb levels (interior and exterior) and blood Pb concentrations. These outcomes support the general concept that contact with Pb in surface dust (e.g., surface dust-to-hand-to-mouth) is a major contributor to Pb intake in children.

One of the largest analyses of relationships between environmental Pb levels and blood Pb concentrations in children was conducted at the Bunker Hill Superfund site, a former Pb mining and smelting site. Although not an epidemiological study, per se, as a part of public health monitoring and remedial investigation studies connected with the site, extensive surveying was conducted of Pb levels in residential soil and interior dust, and blood Pb concentrations in children residing at these residences. These data provide a basis for exploring quantitative relationships between soil and dust exposure variables and blood Pb concentrations, as well as for evaluating mechanistic models that simulate these relationships (see discussion of calibration and evaluation of the IEUBK Model for Lead in Children, Section 4.4.5.2).

TerraGraphics (2000) conducted an analysis of data on environmental Pb levels and child blood Pb concentrations in children, as part of a 5-year review of the clean-up at the Bunker Hill Superfund site, a former Pb mining and smelting site. The analysis included ~4,000 observations of blood Pb concentrations in children between the ages of 9 months and 9 years of age, collected over an 11-year period (1988-1999). The number of children for which blood Pb concentrations were available each year ranged from 230 in 1988 to 445 in 1993; ~54 to 88% of the child population was sampled each year. Blood Pb concentrations (annual geometric mean) ranged from 4.4 to 9.9 µg/dL. Environmental Pb levels (e.g., dust, soil, paint Pb levels) data were collected at ~1300 residences. Interior dust Pb concentrations (annual geometric mean) ranged from ~400 to 4200 ppm. Yard soil Pb concentration (annual geometric mean) ranged from ~100 to 2600 ppm. Several multivariate regression models relating environmental Pb levels and blood Pb concentration were explored; the model having the highest R^2 (0.23) is shown in Table 4-6. The model predicts significant associations between blood Pb concentration, and the (natural log-transformed) community soil Pb concentration ($\beta = 1.76$), neighborhood soil Pb concentration ($\beta = 0.73$; geometric mean soil Pb concentration for areas within 200 ft of the residence), or interior dust Pb concentration ($\beta = 0.84$).

The model predicted a 1.2 µg/dL decrease in blood Pb concentration in association with a decrease in community soil Pb concentration from 2000 to 1000 ppm. The same decrease in neighborhood soil Pb concentration, or interior dust Pb concentration, was predicted to result in a 0.5 or 0.6 µg/dL decrease in blood Pb concentration, respectively. Regression models ($R^2 = 0.86$ to 0.94), based on repeated blood Pb measurements made on the same children from this data set, predicted much stronger associations between current blood Pb concentration and the blood Pb

Table 4-6. General Linear Model Relating Blood Lead Concentration in Children and Environmental Lead Levels—Bunker Hill Superfund Site

Parameter	Coefficient	P-value	Standardized Coefficient
Intercept	−0.22877	0.7947	0.00000
Age (years)	−0.44803	0.0001	−0.25541
ln(interior dust Pb) (ppm)	0.83723	0.0001	0.15677
ln(yard soil Pb) (ppm)	0.21461	0.0080	0.06466
ln(GM soil Pb within 200 ft of residence) (ppm)	0.73100	0.0001	0.12938
ln(GM community soil Pb) (ppm)	1.76000	0.0001	0.19709

$R^2 = 0.231$; $p < 0.0001$; based on data from Bunker Hill Superfund Site collected over the period 1988-1999
GM, geometric mean; ln, natural log

Source: TerraGraphics (2000).

concentration measured in the previous year ($\beta = 0.62$) for the same child than to the corresponding community soil Pb concentration ($\beta = 0.095$) or interior dust Pb concentration ($\beta = 0.1$). Structural equation modeling was applied to the larger data set, utilizing the model structures shown in Figure 4-13. Model 1 included a direct pathway connecting community soil Pb to blood Pb. Both models yielded similar R^2 values (0.89) and predicted a relatively large influence of interior dust Pb on blood Pb (Tables 4-7 and 4-8).

A subsequent analysis was conducted of paired environmental Pb and blood Pb levels in children (n = 126, ages 9 months to 9 years), collected during 1996 to 1998 at various locations in the Coeur d'Alene Basin (outside of the Bunker Hill Site, TerraGraphics, 2001). The annual geometric mean of blood Pb concentrations for the study area was ~4 µg/dL, with the blood Pb concentration range of individuals included in the regression analysis being ~1 to 23 µg/dL. Yard soil Pb concentrations ranged from <100 to 7350 ppm. A model that included all significant ($p \leq 0.05$) variables is shown in Table 4-9. The model predicted a 0.7 µg/dL decrease in blood Pb concentration per 1000 ppm decrease in exterior soil Pb, and a 0.16 µg/dL decrease in blood Pb per 1 mg/cm^2/day decrease in entryway dust Pb loading rate. Entryway dust Pb loading rate was estimated from measurements of the amount of Pb (and dust) recovered from doormats placed at each residence for a known duration. Regression models (general linear

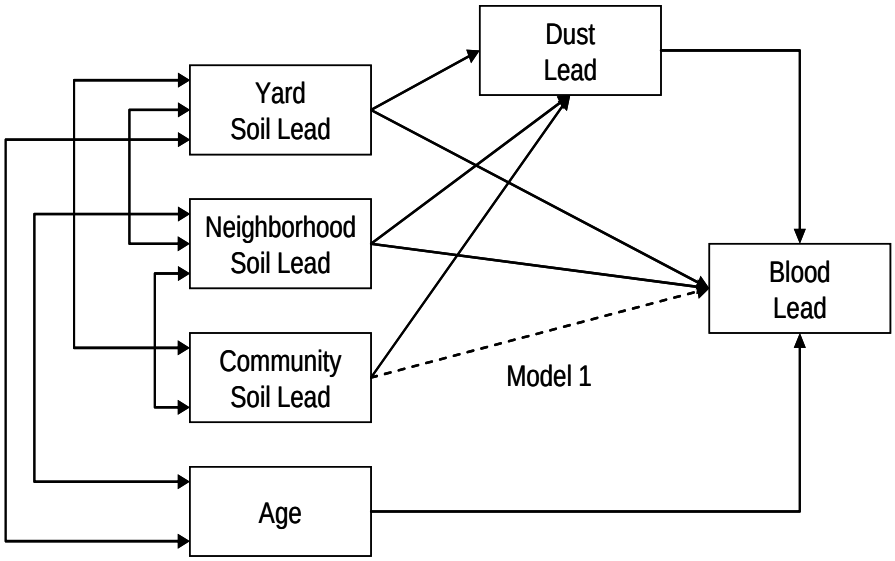

Figure 4-13. Structural equation model for relationships between dust and soil lead and blood lead concentration in children, based on data collected at the Bunker Hill Superfund Site (1988-1999). Neighborhood soil lead is represented in the model as the mean soil lead within 200 feet of the residence, whereas community soil lead is the mean for the city. The pathway between community soil lead and blood lead was included in Model 1 and excluded from Model 2. Units: blood lead, µg/dL; dust and soil lead (µg/g); age, years. See Tables 4-7 and 4-8 for estimated regression coefficients.

Source: TerraGraphics et al. (2000).

model) relating entryway mat Pb loading rate, or mat Pb concentration, and environmental variables were also developed. The strongest predictor of both outcome variables (natural log-transformed) was soil Pb concentration (natural log-transformed, β: ~0.4; R^2 = 0.36-0.46).

Lanphear et al. (1998) conducted a pooled analysis of data on environmental Pb levels and blood Pb concentrations in children (n = 1861) collected as part of 12 epidemiologic studies (conducted over a 15-year period, from 1982 to 1997). Seven of the studies were of communities near Pb mining and/or smelting sites (Bingham Creek, UT; Butte, MT; Leadville, CO; Magna, UT; Midvale, UT; Palmerton, PA; Sandy, UT); and 5 studies were of urban communities (2 in Boston, MA; 2 in Cincinnati, OH; Rochester, NY). The mean age of children included in the analysis was 16 months; the inter-study range was 6 to 24 months. The geometric mean blood Pb concentration for the subjects in the pooled analysis was 5.1 µg/dL;

Table 4-7. Structural Equation Model (1) Relating Blood Lead Concentration in Children and Environmental Lead Levels—Bunker Hill Superfund Site

Parameter	Coefficient	P-value	Standardized Coefficient	Contribution (%)[a]
Model for ln(blood Pb) (µg/dL): $R^2 = 0.892$				
Error	1.000	—	0.329	—
Intercept	−0.519	0.05	−0.171	—
Age (years)	−0.065	0.05	−0.210	—
ln(interior dust Pb) (ppm)	0.159	0.05	0.597	42
ln(yard soil Pb) (ppm)	0.051	0.05	0.171	12
ln(AM soil Pb within 200 ft of residence) (ppm)	0.067	0.05	0.267	19
ln(AM community soil Pb) (ppm)	0.095	0.05	0.389	27
Model for ln(Interior dust Pb) (ppm): $R^2 = 0.986$				
Error	1.000	—	0.117	—
Intercept	3.237	0.05	0.487	—
ln(yard soil Pb) (ppm)	0.129	0.05	0.114	22
ln(AM soil Pb within 200 ft of residence) (ppm)	0.133	0.05	0.141	28
ln(AM community soil Pb) (ppm)	0.235	0.05	0.256	50

Based on data from the Bunker Hill Superfund Site collected over the period 1988 to 1999.
Largest standardized residual, 0.183; Chi-Square, 21.309; P, 0.0001; Comparative fit index, 0.9993; Normed fit index, 0.9993; Non-normed fit index, 0.9863.
GM, geometric mean; ln, natural log

[a]Based on sum of standardized coefficients for dust and soil Pb parameters.

Source: TerraGraphics (2000).

95% were within the range 1.2 to 26 µg/dL and 19% were >10 µg/dL. The geometric mean interior dust Pb loading was 13.5 µg/ft^2 (95% range: 1 to 4500 µg/ft^2) and geometric mean exterior soil or surface dust Pb level was 508 ppm (95% range: 8 to 10,200 ppm). A regression model was developed relating natural log-transformed blood Pb concentration to log-transformed environmental Pb variables, and categorical demographic or behavioral variables (Table 4-10). The R^2 for the final model was 0.53 (uncorrected for measurement error). Measurement error was included in the model as variance estimates for each environmental Pb variable as follows

Table 4-8. Structural Equation Model (2) Relating Blood Lead Concentration in Children and Environmental Lead Levels—Bunker Hill Superfund Site

Parameter	Coefficient	P-value	Standardized Coefficient	Contribution (%)[a]
Model for ln(blood Pb) (µg/dL): $R^2 = 0.892$				
Error	1.000	—	0.329	—
Intercept	−0.206	0.05	−0.116	—
Age (years)	−0.064	0.05	−0.208	—
ln(interior dust Pb) (ppm)	0.165	0.05	0.619	50
ln(yard soil Pb) (ppm)	0.051	0.05	0.171	14
ln(AM soil Pb within 200 ft of residence) (ppm)	0.115	0.05	0.456	37
Model for ln(interior dust Pb) (ppm): $R^2 = 0.986$				
Error	1.000	—	0.117	—
Intercept	3.237	0.05	0.487	—
ln(yard soil Pb) (ppm)	0.129	0.05	0.114	22
ln(AM soil Pb within 200 ft of residence) (ppm)	0.133	0.05	0.141	28
ln(AM community soil Pb) (ppm)	0.235	0.05	0.256	50

Based on data from the Bunker Hill Superfund Site collected over the period 1988 to 1999.
Largest standardized residual, 0.183; Chi-Square, 21.309; P, 0.0001; Comparative fit index, 0.9993; Normed fit index, 0.9993; Non-normed fit index, 0.9863.
AM, arithmetic mean; ln, natural log.
[a]Based on sum of standardized coefficients for dust and soil Pb parameters.

Source: TerraGraphics (2000).

(log-transformed values): dust Pb loading, 1.00; exterior Pb concentration, 1.00; water Pb concentration, 0.75; maximum XRF, 0.75. Of the model variables listed above, significant variables ($p < 0.05$, after correction for measurement error) were as follows: interior dust Pb loading ($\beta = 0.183$, $p < 0.0001$), exterior soil/dust Pb ($\beta = 0.02116$, $p = 0.00025$), age ($\beta = 0.02126$, $p = 0.0044$), mouthing behavior ($\beta = -0.0323$, $p = 0.0004$), and race ($\beta = 0.123$, $p = 0.0079$). Significant interactions in the model included: age and dust Pb loading, mouthing behavior and exterior soil/dust level, and SES and water Pb level. Predicted relationships between interior dust Pb loading or exterior Pb concentrations and blood Pb concentration are shown in Tables 4-11 and 4-12. The model predicted a geometric mean blood Pb concentration

Table 4-9. General Linear Model Relating Blood Lead Concentration in Children and Environmental Lead Levels—Coeur d'Alene Basin

Parameter	Coefficient	P-value	Standardized Coefficient
Intercept	2.8644	0.0032	0.00000
Age (years)	−0.3351	0.0007	−0.2056
Soil Pb (ppm)	0.0007	0.0012	0.2249
Entryway (mat) Pb loading rate ($mg/cm^2/day$)	0.1638	0.0006	0.3212
Median exterior paint Pb (mg/cm^2)	0.5176	0.0005	0.2742
Minimum interior paint condition (categorical: 1-3)	1.9230	0.0008	0.2313

N: 126 (ages 9 mo to 9 years), $R^2 = 0.597$, $p < 0.0001$; based on data from the Coeur d'Alene Basin collected over the period 1996 to 1999.

Source: TerraGraphics (2001).

of 4.0 µg/dL (4% probability of exceeding 10 µg/dL) assuming the study median environmental Pb levels to be as follows: dust Pb, 5.0 $\mu g/ft^2$; soil Pb, 72 ppm; maximum interior paint Pb, 1.6 mg/cm^2; water Pb, 1 ppb.

Succop et al. (1998) conducted a meta-analysis of relationships between environmental Pb levels and blood Pb levels in children (n = 1855, age <72 months) based on data from 11 epidemiologic studies (conducted over a 13-year period, 1981 to 1994). All but 2 of the studies (Cincinnati prospective study, 1981 to 1985; Cincinnati soil Pb study, 1989 to 1991) were of communities near Pb mining and/or smelting sites (Bingham Creek, UT; Butte, MT; Leadville, CO; Magna, UT; Midvale, UT; Palmerton, PA; Sandy, UT; Telluride, CO; Trail, B.C.). The inter-study age range was 15 to 39 months, and the inter-study range of the geometric mean blood Pb concentration was 2.6 to 12.9 µg/dL; 7.5% of children were ≥10 µg/dL. The inter-study geometric mean ranges were: interior dust Pb loading, 31 to 976 $\mu g/m^2$; interior dust Pb concentration, 110 to 1548 ppm; handwipe Pb, 2 to 9 µg; exterior entry dust Pb concentration, 72 to 1830 ppm. Structural equation modeling was applied to the data from each study.

The same generic model was initially applied to each dataset, followed by backward elimination of pathways and co-variables until a model for each study evolved in which all predictors and co-variables were significant ($p \leq 0.05$). The generic model is shown in

Table 4-10. Multivariate Regression Model Relating Blood Lead Concentration in Children and Environmental Lead Levels—Multi-study Pooled Analysis

Parameter	Level	Estimate	P-value
Intercept		1.496	
Dust Pb loading (ug/ft^2)		0.183	<0.0001
Water Pb (ppb)		0.01398	0.2067
Soil or exterior dust Pb (ppm)		0.02116	0.0025
Soil or exterior exposure dust Pb * type of sample		0.005787	0.9247
Soil or exterior exposure dust Pb * type of sample * location		0.4802	0.0409
Type of exterior exposure sample		–0.1336	0.2805
Soil or exterior exposure dust location		0.5858	0.0455
Paint Pb content (mg/cm^3)		–0.02199	0.3402
CLN(MAX XRF) * paint condition		0.03811	0.3888
Paint condition		–0.0808	0.1685
Age		0.02126	<0.0001
Age 2		–0.001399	0.0044
Age 3		0.00007854	0.0022
Study	Boston	–0.3932	<0.0001[a]
	Butte	–0.01167	
	Bingham Creek	0.2027	
	Cincinnati Program	0.2392	
	Cincinnati Soil	0.5383	
	Leadville	0.05717	
	Magna	0.1761	
	Rochester Longitudinal	–0.04209	
	Rochester LID Study	0.07257	
	Sandy	–0.3712	
	Midvale	0.1777	
	Palmerton	0	
Race	Other	0.123	0.0079[a]
	White	0	

Table 4-10. (cont'd). Multivariate Regression Model Relating Blood Lead Concentration in Children and Environmental Lead Levels – Multi-study Pooled Analysis

Parameter	Level	Estimate	P-value
Socioeconomic status (SES)	1	0.3175	0.1081[a]
	2	0.2138	
	3	0.1799	
	4	0.1691	
	5	0	
Mouthing behavior	Often	–0.03233	0.0004[a]
	Rarely	–0.2454	
	Sometimes	–0.1397	
	Unknown	0	
Dust Pb loading * Age		0.002649	0.1860
Dust Pb loading * Age 2		–0.0003381	0.0573
Dust Pb loading * Age 3		–0.00001281	0.6185
Exterior Pb exposure * mouthing behavior	Often	0.2212	0.0419[a]
	Rarely	0.07892	
	Sometimes	0.1663	
	Unknown	0	
Water Pb levels (ppb) * SES	1	0.5305	0.0998[a]
	2	–0.0136	
	3	0.1033	
	4	–0.09098	
	5	0	
Age * race	Other	0.01192	0.0129[a]
	White	0	
Age * SES	1	–0.01023	0.0061[a]
	2	0.003849	
	3	0.00008468	
	4	–0.01679	
	5	0	
Standard deviation of the prediction error		0.5425	

Interactions are indicated by asterisks. Blood Pb concentration (µg/dL) and all environmental Pb variables were natural log-transformed. R^2 for blood Pb concentration was 0.53 (uncorrected for measurement error).

[a]Overall factor significance.

Source: Lanphear et al. (1998).

Table 4-11. Children's Predicted Blood Lead Levels for Floor Dust Lead Loading (µg/ft^2) and Exterior Lead Exposures (ppm)[a]

Dust Pb loading (µg/ft^2)	Geometric mean blood Pb levels (µg/dL) with 90% Confidence Intervals[a] in parentheses							
	Exterior Pb exposure (ppm)							
	10	72[b]	100	500	1000	1500	2000	4000
1	2.3 (0.9, 5.7)	2.8 (1.1, 7.0)	2.9 (1.2, 7.3)	3.5 (1.4, 8.7)	3.8 (1.5, 9.4)	4.0 (1.6, 9.8)	4.1 (1.6, 10.1)	4.4 (1.8, 11.0)
5	3.2 (1.3, 8.0)	4.0 (1.6, 9.8)	4.1 (1.7, 10.1)	4.9 (2.0, 12.0)	5.3 (2.1, 13.0)	5.5 (2.2, 13.6)	5.7 (2.3, 14.0)	6.1 (2.5, 15.2)
10	3.7 (1.5, 9.2)	4.6 (1.8, 11.3)	4.7 (1.9, 11.7)	5.6 (2.3, 13.9)	6.1 (2.5, 15.0)	6.3 (2.6, 15.7)	6.5 (2.7, 16.2)	7.1 (2.9, 17.5)
15	4.0 (1.6, 10.0)	5.0 (2.0, 12.3)	5.1 (2.1, 12.7)	6.1 (2.5, 15.1)	6.6 (2.7, 16.3)	6.9 (2.8, 17.0)	7.1 (2.9, 17.6)	7.7 (3.1, 19.0)
20	4.2 (1.7, 10.6)	5.3 (2.1, 13.0)	5.4 (2.2, 13.5)	6.5 (2.6, 16.0)	7.0 (2.8, 17.3)	7.3 (3.0, 18.0)	7.6 (3.1, 18.6)	8.1 (3.3, 20.1)
25	4.4 (1.8, 11.2)	5.5 (2.2, 13.6)	5.7 (2.3, 14.1)	6.8 (2.8, 16.8)	7.3 (3.0, 18.1)	7.7 (3.1, 18.9)	7.9 (3.2, 19.5)	8.5 (3.5, 21.1)
40	4.9 (1.9, 12.3)	6.1 (2.4, 15.0)	6.3 (2.5, 15.6)	7.5 (3.0, 18.5)	8.1 (3.3, 19.9)	8.4 (3.4, 20.8)	8.7 (3.5, 21.5)	9.4 (3.8, 23.2)
55	5.2 (2.1, 13.2)	6.5 (2.6, 16.1)	6.7 (2.7, 16.6)	8.0 (3.2, 19.7)	8.6 (3.5, 21.3)	9.0 (3.7, 22.2)	9.3 (3.8, 22.9)	10.0 (4.1, 24.8)
70	5.5 (2.2, 13.8)	6.8 (2.7, 16.9)	7.0 (2.8, 17.5)	8.4 (3.4, 20.7)	9.1 (3.7, 22.3)	9.5 (3.8, 23.4)	9.8 (4.0, 24.1)	10.5 (4.3, 26.0)
100	5.9 (2.3, 14.9)	7.3 (2.9, 18.2)	7.6 (3.1, 18.9)	9.0 (3.7, 22.3)	9.7 (3.9, 24.1)	10.2 (4.1, 25.2)	10.5 (4.3, 26.0)	11.3 (4.6, 28.0)

[a]Confidence interval is estimated to cover 90% of the observed blood Pb levels with 5% above and 5% below the interval.
[b]Values for an exterior Pb paint exposure of 72 ppm were estimated median levels based on U.S. Housing and Urban Development national survey, 1989-1990

Source: Lanphear et al. (1998).

Figure 4-14, along with the percent of studies in which a given pathway was found to be significant. The most common exposure pathway influencing blood Pb concentration (i.e., significant in models of most studies) was exterior soil, operating through its effect on interior dust Pb and hand Pb. Paint Pb was also a significant influential variable on the soil and interior dust-to-blood pathway in ~40% of the studies. Significant co-variables varied across studies and included: child age, mouthing frequency, time spent outdoors, SES, house age and condition, home renovation, parental occupation, bare soil in yard, and presence of pets.
The relative strength of the influence of various environmental sources of Pb in the structural

Table 4-12. Likelihood of a Child's Blood Lead ≥10 µg/dL for Floor Dust Lead Loadings and Exterior Exposure Levels (ppm)[a]

Dust Pb loading (µg/ft^2)	Probability of blood Pb greater than 10 µg/dL							
	Exterior Pb exposure (ppm)							
	10	72[b]	100	500	1000	1500	2000	4000
1	0.33% (0.05, 2.24)	1.0% (0.3, 3.8)	1.2% (0.3, 4.2)	2.7% (0.9, 7.4)	3.7% (1.3, 9.7)	4.4% (1.6, 11.5)	4.9% (1.8, 12.8)	6.5% (2.3, 16.9)
5	1.8% (0.4, 7.9)	4.4% (1.7, 11.0)	5.0% (2.0, 11.8)	9.3% (4.7, 17.6)	12% (6, 21)	14% (7, 24)	15% (8, 26)	18% (9, 32)
10	3.3% (0.8, 12.6)	7.4% (3.1, 16.5)	8.3% (3.8, 17.5)	14% (8, 24)	18% (10, 29)	20% (12, 32)	22% (13, 35)	26% (15, 41)
15	4.5% (1.2, 16.2)	9.8% (4.3, 20.7)	11% (5, 22)	18% (11, 29)	22% (14, 34)	25% (15, 37)	27% (16, 40)	31% (19, 47)
20	5.7% (1.5, 19.2)	12% (5, 24)	13% (6, 25)	21% (13, 33)	26% (16, 38)	28% (18, 41)	30% (19, 44)	35% (22, 51)
25	6.7% (1.8, 21.8)	14% (6, 27)	15% (7, 28)	24% (15, 36)	28% (18, 41)	31% (20, 45)	33% (22, 47)	38% (25, 54)
40	9.4% (2.7, 27.8)	18% (9, 33)	20% (10, 35)	30% (19, 43)	35% (23, 48)	38% (25, 52)	40% (27, 54)	45% (31, 61)
55	12% (3, 32)	21% (10, 38)	23% (12, 40)	34% (22, 48)	39% (27, 53)	42% (29, 57)	45% (31, 59)	50% (35, 65)
70	13% (4, 36)	24% (12, 42)	26% (14, 44)	37% (24, 52)	43% (29, 57)	46% (32, 60)	48% (34, 63)	54% (38, 69)
100	17% (5, 41)	28% (14, 48)	31% (16, 49)	43% (28, 58)	48% (34, 63)	51% (37, 66)	54% (39, 68)	59% (43, 73)

[a]All other variables held at their national median.
[b]Estimated median levels based on U.S. Housing and Urban Development national survey, 1989 to 1990.

Source: Lanphear et al. (1998).

equation model, on blood Pb concentration, was evaluated by applying a simple linear regression model to the geometric mean values for environmental variables (natural log-transformed) and blood Pb concentrations (natural log-transformed), from the individual studies (Table 4-13). The strongest relationships were obtained for interior dust Pb loading ([$\beta = 0.474$, R^2: 0.96] and handwipe Pb [$\beta = 1.184$, $R^2 = 0.90$]). The models predicted a 8.6 µg/dL decline in blood Pb concentration (from ~15 µg/dL) for a 1000 µg/cm^2 reduction in interior dust Pb loading (from 1100 µg/cm^2), and a 14.4 µg/dL decline in blood Pb concentration (from 10 µg) for a 10 µg reduction in handwipe Pb.

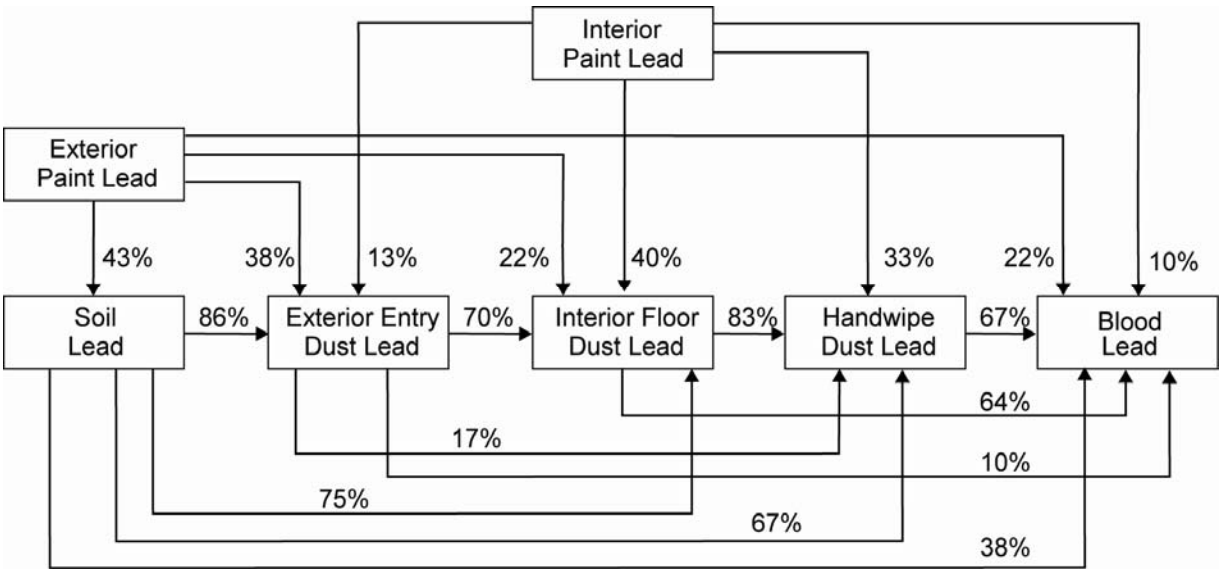

Figure 4-14. Structural equation model for relationships between dust and soil lead and blood lead concentration in children. Numbers are the percentage of 11 studies included meta-analysis for which the pathway was significant (p = 0.05). Units: blood lead, µg/dL; dust and soil lead, µg/g; handwipe lead (µg); pant lead, mg/cm^2

Source: Succop et al. (1998).

Lanphear and Roghmann (1997) collected data on blood Pb concentrations for 205 children residing in Rochester, NY (1991-1992) paired with their residential environmental Pb levels. The mean age of the children was 20 months (range: 12 to 30 months). Mean blood Pb concentration was 7.7 µg/dL (SD: 5.1), with 23% of children having a blood Pb ≥10 µg/dL. Geometric mean interior dust Pb loading was 106 µg/ft^2 (±SD: 10, 1167) and soil Pb level was 981 ppm (±SD: 225, 4267). Data on the following variables were used for structural equations modeling: serum ferritin (ng/dL), blood Pb concentration (µg/dL), hand Pb (µg), interior dust Pb loading (µg/ft^2), paint Pb loading (XRF, mg/cm^2), water Pb (ppb), soil Pb (ppm), race, parent marital status, household income, maternal cleaning behaviors, and child exposure behaviors (e.g., time spent outside, mouthing, dirt ingestion). A structural equation model, shown in Figure 4-15, yielded an R^2 of 0.41 for blood Pb concentration. The exposure pathway most influential on blood Pb was interior dust Pb loading, directly or through its influence on hand Pb. Both soil and paint Pb influenced interior dust Pb; with the influence of paint Pb greater than that of soil

Table 4-13. Meta-analysis of the Relationship Between Log-transformed Blood Lead and Various Environmental Lead Sources[a]

Independent variable	Units	Intercept	Slope Estimate	Squared Correlation	No. of Studies	Predicted Decline in Blood Lead[b]
In(handwipe Pb)	µg	0.009	1.184	0.90	6	14.4
In(interior dust Pb loading)	µg/m^2	–0.479	0.444	0.55	10	9.1
In(interior dust Pb loading)[c]	µg/m^2	–0.782	0.474	0.96	8	8.6
In(interior dust Pb concentration)	ppm	–1.502	0.529	0.58	10	6.5
In(exterior entry dust Pb concentration)	ppm	–1.101	0.435	0.72	10	4.5
In(perimeter soil Pb concentration)	ppm	–0.015	0.233	0.65	6	2.2
In(maximum interior paint Pb loading)	mg/cm^2	1.562	0.232	0.07	8	2.1
In(maximum exterior paint Pb loading)	mg/cm^2	1.502	0.152	0.07	9	1.3

[a]These are simple relationships unadjusted for covariates.
[b]Predicted decline in blood Pb for a reduction in hand Pb of 10–1 µg; dust Pb loading of 1100 to 100 µg/m^2; dust Pb or soil Pb concentration of 1100–100 ppm; or paint Pb loading of 3.0–0.5 mg/cm^2 as calculated from the fitted linear regression equation: In(blood Pb) = intercept + slope x In(environmental Pb).
[c]Excluding the Trail and Cincinnati soil project studies, which appear to be outliers. The exposure in these two studies appears to be primarily from exterior dust Pb.

Source: Succop et al. (1998).

Pb. Other influential variables were Black race (direct), family income (direct), and outside play (indirect) through dirt ingestion behavior. Simple correlation analysis also revealed relatively strong (significant, $p < 0.5$) associations between dust Pb loading ($r = 0.41$), soil Pb concentration (0.31) and Black race ($r = 0.44$).

Bornschein et al. (1985a) applied structural equation modeling to paired environmental Pb and blood Pb data collected on a subset of children (n = 45) from the Cincinnati Prospective Study (1981-1985). The age range of children included in the study was 9 to 24 months. Group statistics for the blood Pb concentrations of children included in the analysis were not reported in Bornschein et al. (1985a); however, a subsequent analysis of data from the study reported a geometric mean of 12.9 µg/dL (n = 149; Succop et al. 1998). Similarly, dust and soil Pb levels

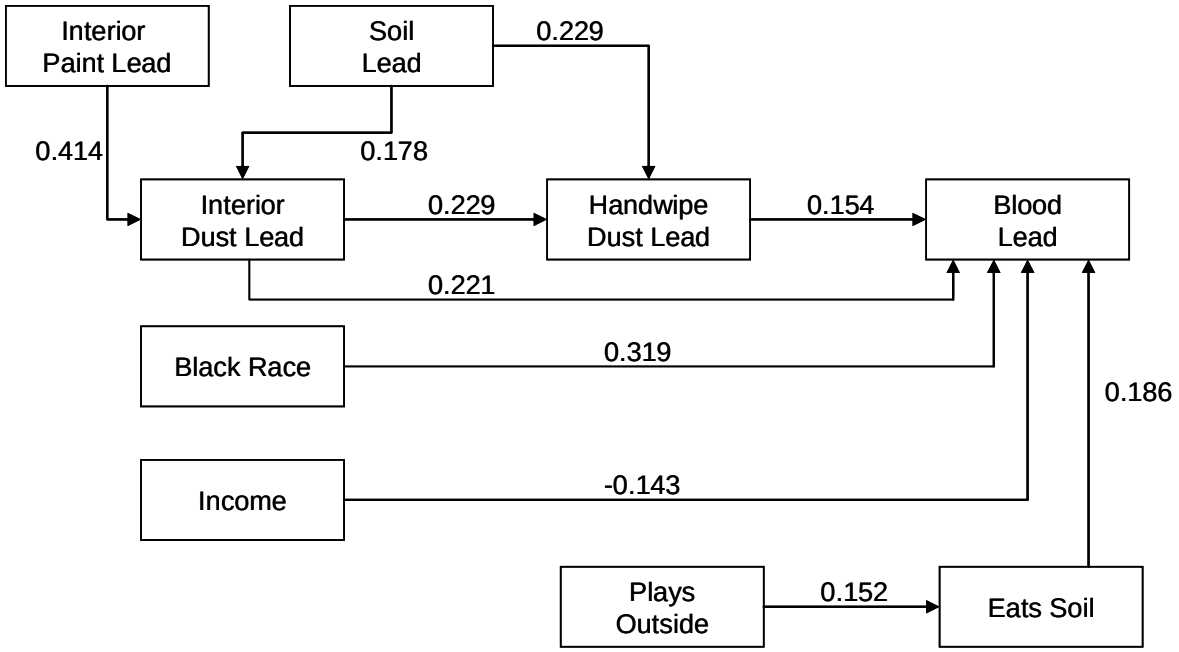

Figure 4-15. Structural equation model for relationships between dust and soil lead and blood lead concentration in children, based on data collected in the Rochester (NY) Lead in Dust Study. Numbers are model coefficients. Units: blood lead, µg/dL; dust lead µg/ft^2; soil lead, µg/g; handwipe lead (µg); pant lead, mg/cm^2, plays outside, categorical: 0-1; eats soil, categorical: 0-1. R^2 values: blood lead, 0.41; hand lead Pb, 0.14; dust lead, 0.25.

Source: Lanphear and Roghmann (1997).

were not reported in Bornschein et al. (1985a), but were reported in Succop et al. (1998) for a larger study group (n = 149) as follows (geometric means): interior dust Pb loading, 976 µg/m^2; interior dust Pb concentration, 1548 ppm; handwipe Pb, 7 µg; and exterior entry dust Pb concentration, 1830 ppm. A structural equation model (Bornschein et al., 1985a), shown in Figure 4-16, yielded R^2 values that ranged from 0.44 to 0.59 across age groups from 9 (R^2 = 0.59) to 24 months (R^2 = 0.44). The exposure pathway most influential on blood Pb was interior dust Pb concentration, directly or through its influence on hand Pb (exterior soil Pb concentration and internal paint Pb was excluded from the model, as was race). Blood Pb concentration was also influenced directly by SES. Interior dust Pb loading was influenced by housing condition variables. Hand dust was directly influenced by maternal involvement with

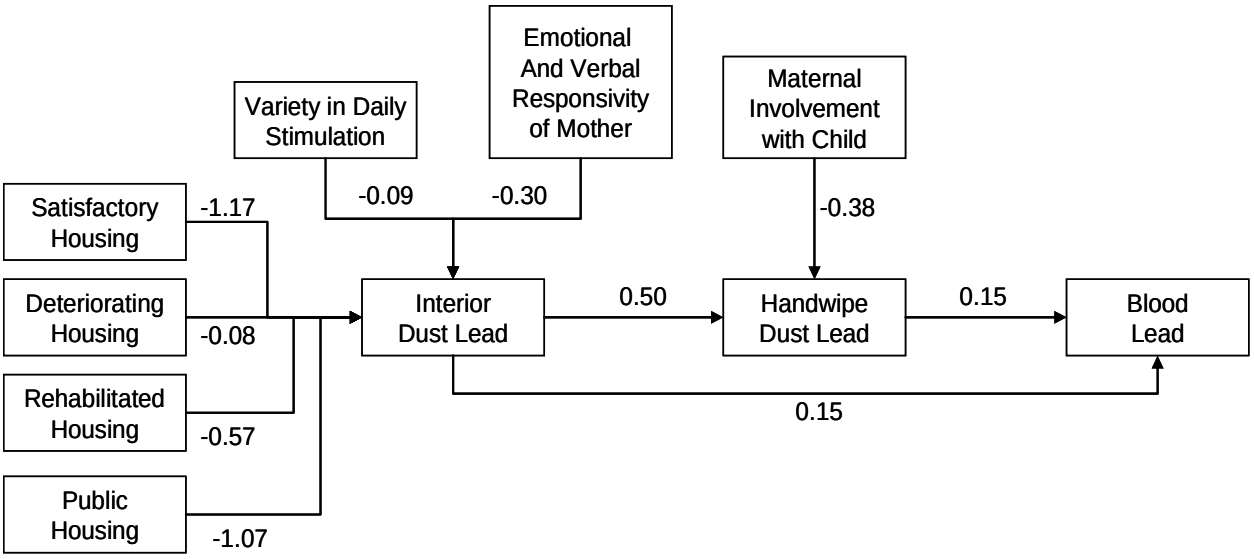

Figure 4-16. Structural equation model for relationships between dust and soil lead and blood lead concentration in children, based on data collected in the Cincinnati (OH) Prospective Child Study. Numbers are model coefficients. Units: blood lead, µg/dL; dust and soil lead, µg/g; handwipe lead (µg); pant lead, mg/cm^2; maternal involvement, categorical: 0-6; responsivity of mother, categorical: 0-11; variety in daily stimulation, categorical: 0-5; housing characteristics, categorical: 0-1. R^2 values: blood lead, 0.41; hand lead, 0.14; dust lead, 0.25.

Source: Bornschein et al. (1985a).

the child. Based on the above model, the relationship between blood Pb concentration, interior dust Pb, and hand Pb, at 18 months of age, was as follows:

$$PbB = 1.94 - 0.02(SES) + 0.15(PbD) + 0.15(PbH) \quad (4\text{-}10)$$

$$PbH = 0.52 - 0.36(material\ involvement) + 0.50(PbD) \quad (4\text{-}11)$$

where PbB, PbD, PbH are the natural log-transformed blood Pb concentration (µg/dL), dust Pb concentration (ppm), and hand Pb (µg), respectively. The above relationship predicts a decline in blood Pb concentration ranging from 8 µg/dL (maternal involvement score, 6) to 11 µg/dL (maternal involvement score, 0), for a reduction in interior dust Pb concentration from 1100 ppm

to 100 ppm (assuming SES score of 17, based on geometric mean reported for the Cincinnati child study in Succop et al. 1998).

The Urban Soil Lead Abatement Demonstration Project (USLADP) was a study conducted to determine if urban soil Pb abatement would affect the Pb exposures and blood Pb concentrations of urban children (U.S. Environmental Protection Agency, 1996b). The study included measurement of blood Pb concentrations and environmental Pb prior to and following removal of Pb-contaminated soils and surface dusts from selected urban neighborhoods in Baltimore (Farrell, 1988), Boston (Aschengrau et al., 1994; Weitzman et al., 1993) and Cincinnati (Clark et al., 1988, 1991, 1996). The numbers of children included in each study were ~182 in the Baltimore study, 92 in the Boston study, and 169 in the Cincinnati study. Pre-abatement blood Pb concentrations (geometric mean) were ~11 µg/dL in the Baltimore study, 12 µg/dL in the Boston study, and 10 µg/dL in the Cincinnati study. Pre-abatement soil and interior floor dust Pb concentrations (geometric mean), respectively, were ~420 and 1700 ppm in the Baltimore study; 2300 and 2200 ppm in the Boston study; and 400 and 300 ppm in the Cincinnati study. Measurements of paired environmental Pb levels and blood Pb concentrations provided the basis for the development of regression models relating blood Pb concentration to Pb levels in interior dust and exterior soil. An extensive analysis of the data collected in each study is reported in U.S. Environmental Protection Agency (1996b), from which selected examples are provided here.

Structural equation modeling was applied to the data from the Boston and Cincinnati studies; the generic model applied to these data is shown in Figure 4-17 and parameters for selected models (based on cross-sectional data) are presented in Table 4-14. The model based on the Cincinnati data showed a stronger association between interior dust Pb and blood Pb concentration, compared to the model based on the data from the Boston study. Soil Pb level influenced blood Pb concentration directly and secondarily, through its influence on interior dust. Repeated measure models and longitudinal structural equation models were also developed based on these data, and are described in detail in U.S. Environmental Protection Agency (1996b).

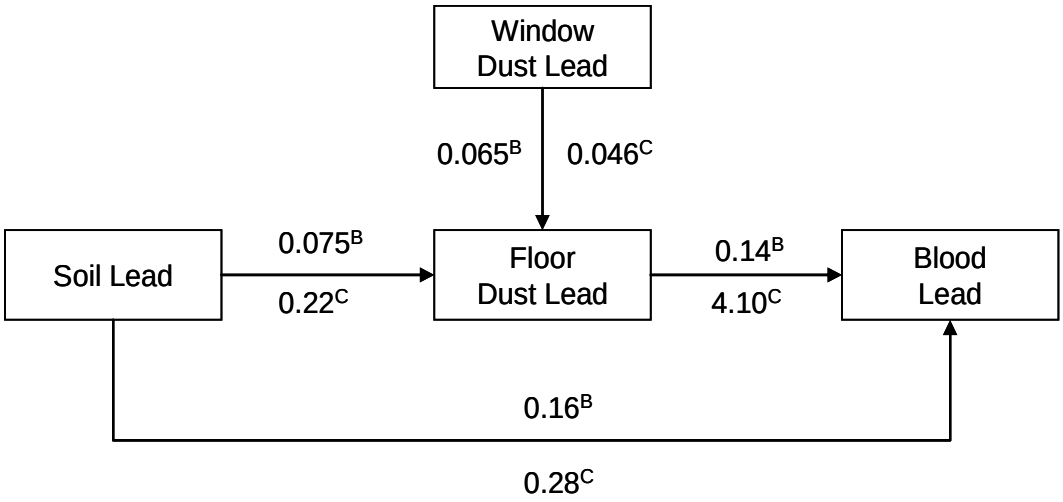

Figure 4-17. Structural equation model for relationships between dust and soil lead and blood lead concentration in children, based on pre-abatement cross-sectional data collected in the Urban Soil Lead Abatement Demonstration Project. Numbers are model coefficients for the Boston study (B) or Cincinnati study (C). Units: blood, µg/dL; dust, soil lead, µg/g; blood coefficient, µg/dL lead in blood per 1000 µg/g lead in soil.

Source: U.S. Environmental Protection Agency (1996b).

4.4.4 Historic Overview of Mechanistic Models of Lead Biokinetics

4.4.4.1 Rabinowitz Model

Early Pb modeling applications presented Pb biokinetics in classical pharmacokinetics terms. Compartments represented kinetically homogeneous pools of Pb which might be associated with individual organs or groups of organs. Among the first of such models was one proposed by Rabinowitz et al. (1976) based on a study of the kinetics of ingested stable Pb isotope tracers and Pb mass balance data in five healthy adult males (Figure 4-18). The Rabinowitz model has three compartments: (1) a central compartment representing blood and other tissues and spaces in rapid equilibrium with blood (e.g., interstitial fluid); (2) a shallow tissue compartment, representing soft tissues and rapidly exchanging pools within the skeleton; and (3) a deep tissue compartment, representing, primarily, slowly exchanging pools of Pb within bone. Excretion pathways include urinary (from the central compartment) and bile,

Table 4-14. Structural Equation Models Relating Blood Lead Concentration in Children and Pre-abatement Environmental Lead Levels—Lead in Urban Soil Abatement Demonstration Project

Parameter	Boston Study	Cincinnati Study
	Model for blood Pb (µg/dL)	
Intercept	10.97[a]	7.55[a]
Floor dust Pb (ppm)	0.14	4.10[d]
Soil Pb (ppm)	0.16	0.28
	Model for floor dust Pb (µg/g)	
Intercept	1008[a]	99.9[b]
Soil Pb (ppm)	0.075	0.2247[c]
Window dust Pb (ppm)	0.0651[a]	0.0458[c]

N = 126 (ages 9 mo to 9 years), $R^2 = 0.597$, $P < 0.0001$; based on data from the Urban Soil Lead Abatement Demonstration Project. Blood coefficients are expressed as µg/dL per µg/g; floor dust Pb coefficients are expressed as µg/g per µg/g.

[a] P = <0.0001
[b] P = 0.0002-0.0019
[c] P = 0.002-0.0099
[d] P = 0.01-0.0499

Source: U.S. Environmental Protection Agency (1986).

sweat, hair, and nails (from the shallow tissue compartment). The model predicts pseudo-first order half-times for Pb of ~25, 28, and 7000 days in the central, shallow tissue, and deep compartments, respectively (these values were calculated based on reported residence times, the reciprocal of the sum of the individual elimination rate constants). The slow kinetics of the deep tissue compartment led to the prediction that it would contain most of the Pb burden following chronic exposures (e.g., for years), consistent with Pb measurements made in human autopsy samples (Barry, 1975; Gross et al., 1975; Schroeder and Tipton, 1968). Note that this model did not simulate the distribution of Pb within blood (e.g., erythrocytes and plasma), nor did it simulate subcompartments within bone or physiological processes of bone turnover that might affect kinetics in the deep tissue compartment.

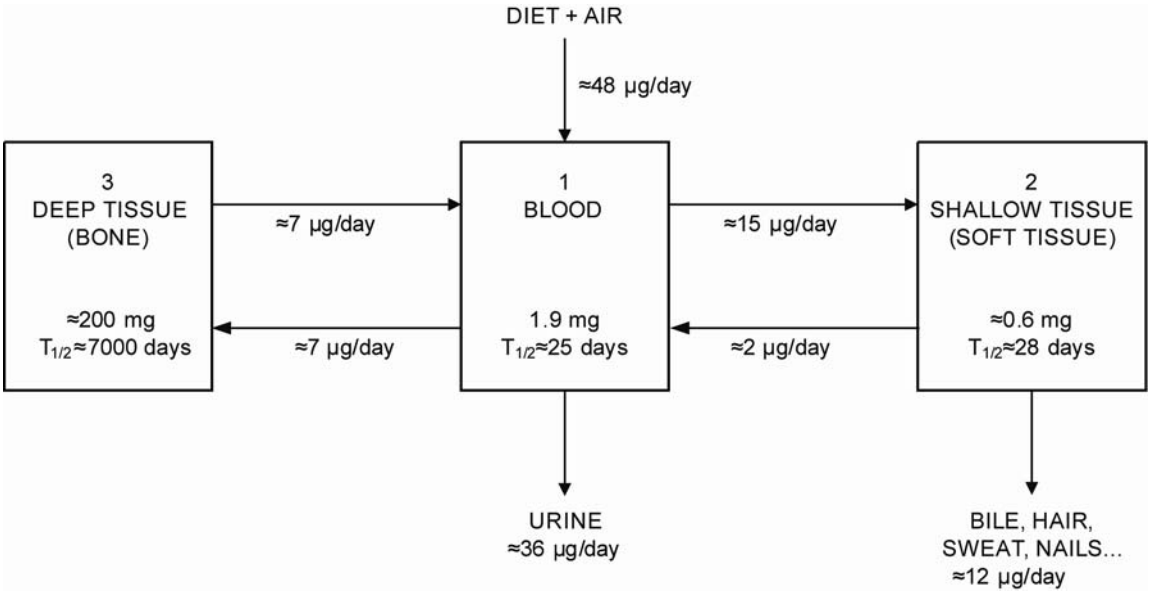

Figure 4-18. Lead biokinetics based on Rabinowitz et al. (1976). Half-times are based on reported mean residence times for compartments 1, 2, and 3: 36, 40, and 10^4 days, respectively (half-time = 0.693*residence time).

4.4.4.2 Marcus Model(s)

Marcus (1985a) reanalyzed the data from stable isotope tracer studies of Rabinowitz et al. (1976) and derived an expanded multicompartment kinetic model for Pb (Figure 4-19). The model included separate compartments with different Pb turnover rates for cortical (slow, t1/2 = 1.2×10^4 to 3.5×10^4 days) and trabecular (fast, t1/2 = 100 to 700 days) bone, an approach subsequently adopted in several other models (O'Flaherty, 1995; U.S. Environmental Protection Agency, 1994a,b; Leggett, 1993; O'Flaherty, 1993; Bert et al., 1989). A more complex representation of the Pb disposition in bone included explicit simulation of Pb diffusion within the bone volume of the osteon and exchange with blood at the canaliculus (Marcus, 1985b; Figure 4-20). Lead diffusion in bone was based on Pb kinetics data from studies conducted in dogs. A similar approach to simulating radial diffusion of Pb in bone, expanded to include eight concentric diffusion shells, was implemented by O'Flaherty (1993, 1995). Marcus (1985c) also introduced nonlinear kinetics of exchange of Pb between plasma and erythrocytes. The blood kinetics included four blood subcompartments: diffusible Pb in plasma, protein-bound Pb in plasma, a "shallow" erythrocyte pool, and a "deep" erythrocyte pool (see Figure 4-21).

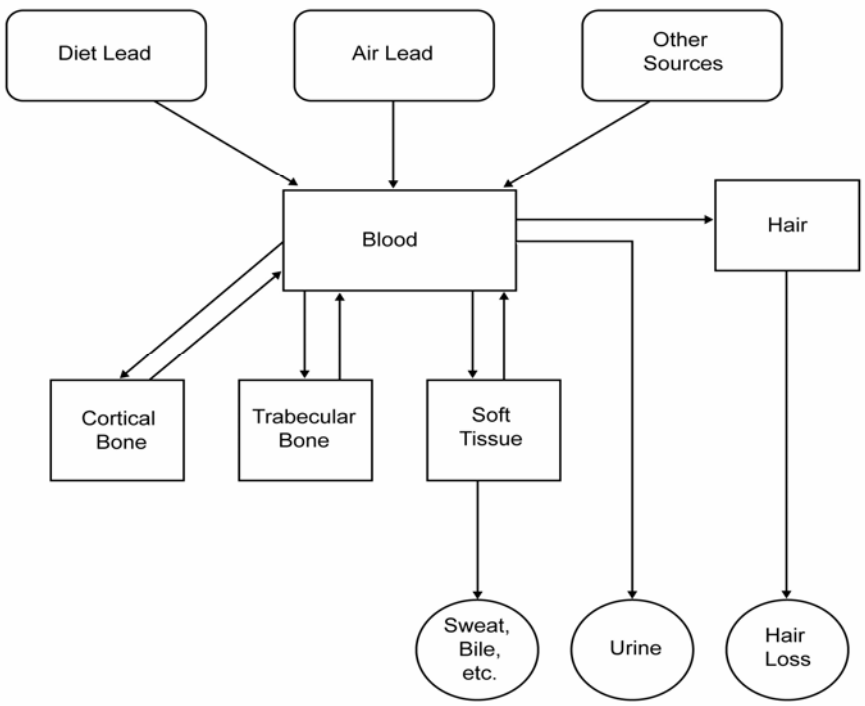

Figure 4-19. Lead biokinectics based on Marcus (1985a). Bone is represented as a slow turnover (cortical) compartment and a faster (trabecular) compartment.

The Marcus (1985c) model predicted the curvilinear relationship between plasma and blood Pb concentrations that has been observed in humans (DeSilva, 1981).

4.4.4.3 Bert Model

Bert et al. (1989) adopted the bone model from Marcus (1985a), in which the bone compartment is subdivided into slow cortical bone and faster trabecular bone compartments (Figure 4-22). The central compartment (denoted as *blood*) is assumed to be 1.5 times the volume of whole blood (Rabinowitz et al., 1976), with the whole blood volume varying in direct proportion with body weight. The model includes a discrete pathway for excretion of unabsorbed Pb from the GI tract into feces. Secretion of Pb in bile, gastric secretions, and saliva are represented as transfers from the soft tissue compartment to the GI tract. Compartment transfer coefficients were based on average values estimated for four individuals from the

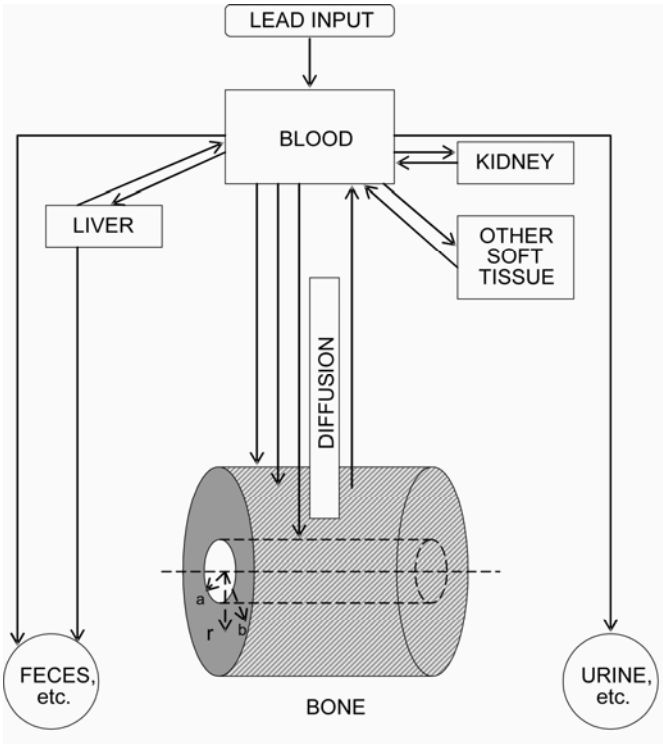

Figure 4-20. Lead biokinetics based on Marcus (1985b). Bone is represented as an extended cylindrical canalicular territory. The canalicular territory has a radius b, and surrounds the canaliculus of radius a. Lead diffuses across radius library, between the fluid in the canaliculus (which is in communication with blood in the Haversian canal, not shown) and the bone volume of the canalicular territory.

Rabinowitz et al. (1976) study. Initial average values for Pb in cortical bone for a given age at the start of a simulation were derived from Barry (1975).

4.4.4.4 Contemporary Models

Additional information on Pb biokinetics, bone mineral metabolism, and Pb exposures has led to further refinements and expansions of these earlier modeling efforts. In particular, three pharmacokinetic models are currently being used or are being considered for broad application in Pb risk assessment: (1) the Integrated Exposure Uptake BioKinetic (IEUBK) model for Pb in children developed by EPA (U.S. Environmental Protection Agency, 1994a,b; White et al., 1998); (2) the Leggett model, which simulates Pb kinetics from birth through adulthood

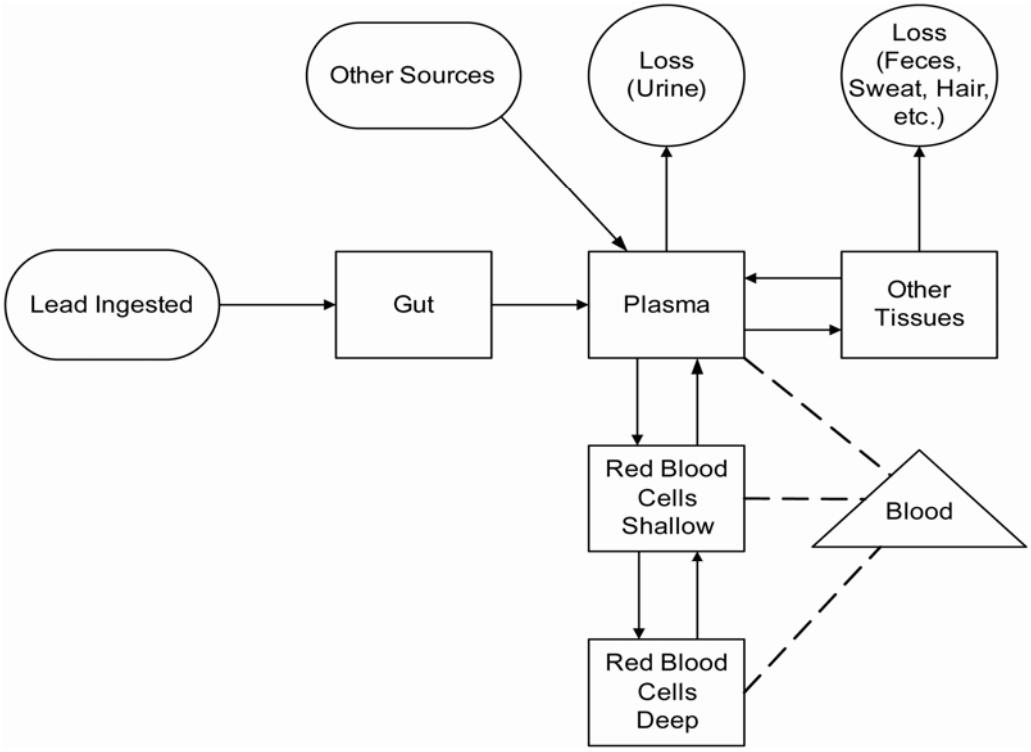

Figure 4-21. Lead biokinetics based on Marcus (1985c). Blood is represented with a plasma (central exchange) compartment and a red blood cell compartment, the latter having shallow and deep pools.

(Leggett, 1993); and (3) the O'Flaherty model, which simulates Pb kinetics from birth through adulthood (O'Flaherty, 1993, 1995). Of the three approaches, the O'Flaherty model has the fewest Pb-specific parameters and relies more extensively on physiologically based parameters to describe volumes, flows, composition, and metabolic activity of blood and bone that determine the disposition of Pb in the human body. Both the IEUBK model and the Leggett model are more classical multicompartmental models; that is, the values for the age-specific transfer rate constants for Pb are based on kinetics data obtained from studies conducted in animals and humans and may not have precise physiological correlates. Thus, the structure and parameterization of the O'Flaherty model is distinct from both the IEUBK model and Leggett model. All three models represent the rate of uptake of Pb (i.e., amount of Pb absorbed per unit of time) as relatively simple functions (f) of Pb intake:

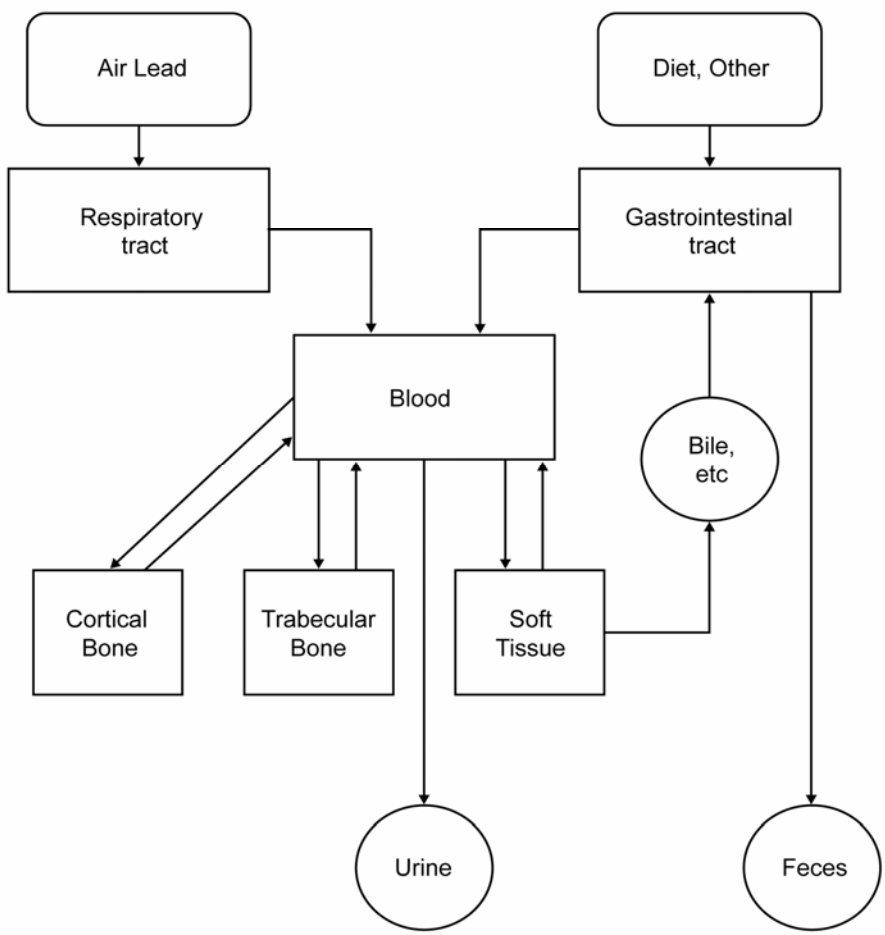

Figure 4-22. Lead biokinetics based on Bert et al. (1989).

$$Uptake = Intake \cdot AF \tag{4-12}$$

$$Uptake = Intake \cdot f_{(Intake)} \tag{4-13}$$

Values assigned to absorption factor (AF) or other variables in $f(_{Intake})$ are, in general, age-specific and environmental medium-specific in some models. However, the models do not modify the representation of uptake as functions of the many other physiologic variables that may affect Pb absorption (e.g., nutritional status). While one can view this approach as a limitation of the models, it also represents a limitation of the data available to support more complex representations of Pb absorption.

The IEUBK model simulates multimedia exposures, uptake, and kinetics of Pb in children ages 0 to 7 years; the model is not intended for use in predicting Pb pharmacokinetics in adults. The O'Flaherty and Leggett models are lifetime models, and include parameters that simulate uptake and kinetics of Pb during infancy, childhood, adolescence, and adulthood. Lead exposure (e.g., residence-specific environmental Pb concentrations, childhood activity patterns) is not simulated by current versions of the O'Flaherty and Leggett models; however, this is not necessarily a limitation since existing exposure models can be used to derive exposure inputs (in terms of Pb intakes) for these models. By contrast, the IEUBK model includes parameters for simulating exposures and uptake to estimate daily uptake of Pb (µg/day) among populations of children potentially exposed via soil and dust ingestion, air inhalation, Pb-based paint chip ingestion, tap water ingestion, and/or diet.

The above three models have been individually evaluated, to varying degrees, against empirical physiological data on animals and humans and data on blood Pb concentrations in individuals and/or populations (U.S. Environmental Protection Agency, 1994a,b; Leggett, 1993; O'Flaherty, 1993). However, applications in risk assessment typically require that the models accurately predict blood Pb distributions in real populations (U.S. Environmental Protection Agency, 1994a), in particular those values or percentages falling in the "upper tails" (e.g., ≥95th percentiles) of the distributions, when input to the models consists of data that describe site-specific exposure conditions, e.g., environmental Pb concentrations or physicochemical properties of soil and dust (Beck et al., 2001; Griffin et al., 1999a,b). In evaluating models for use in risk assessment, exposure data collected at hazardous waste sites have been used to drive model simulations (Bowers and Mattuck, 2001; Hogan et al., 1998). The exposure module in the IEUBK model makes this type of evaluation feasible.

4.4.5 Integrated Exposure Uptake Biokinetic (IEUBK) Model for Lead in Children

4.4.5.1 Model Structure

The IEUBK model for Pb in children (see Figure 4-23) is a multicompartmental pharmacokinetics model linked to an exposure and probabilistic model of blood Pb concentration distributions in children (U.S. Environmental Protection Agency, 1994a,b; White et al., 1998). The model simulates exposure and biokinetics of Pb from birth to age 7 years (84 months) and

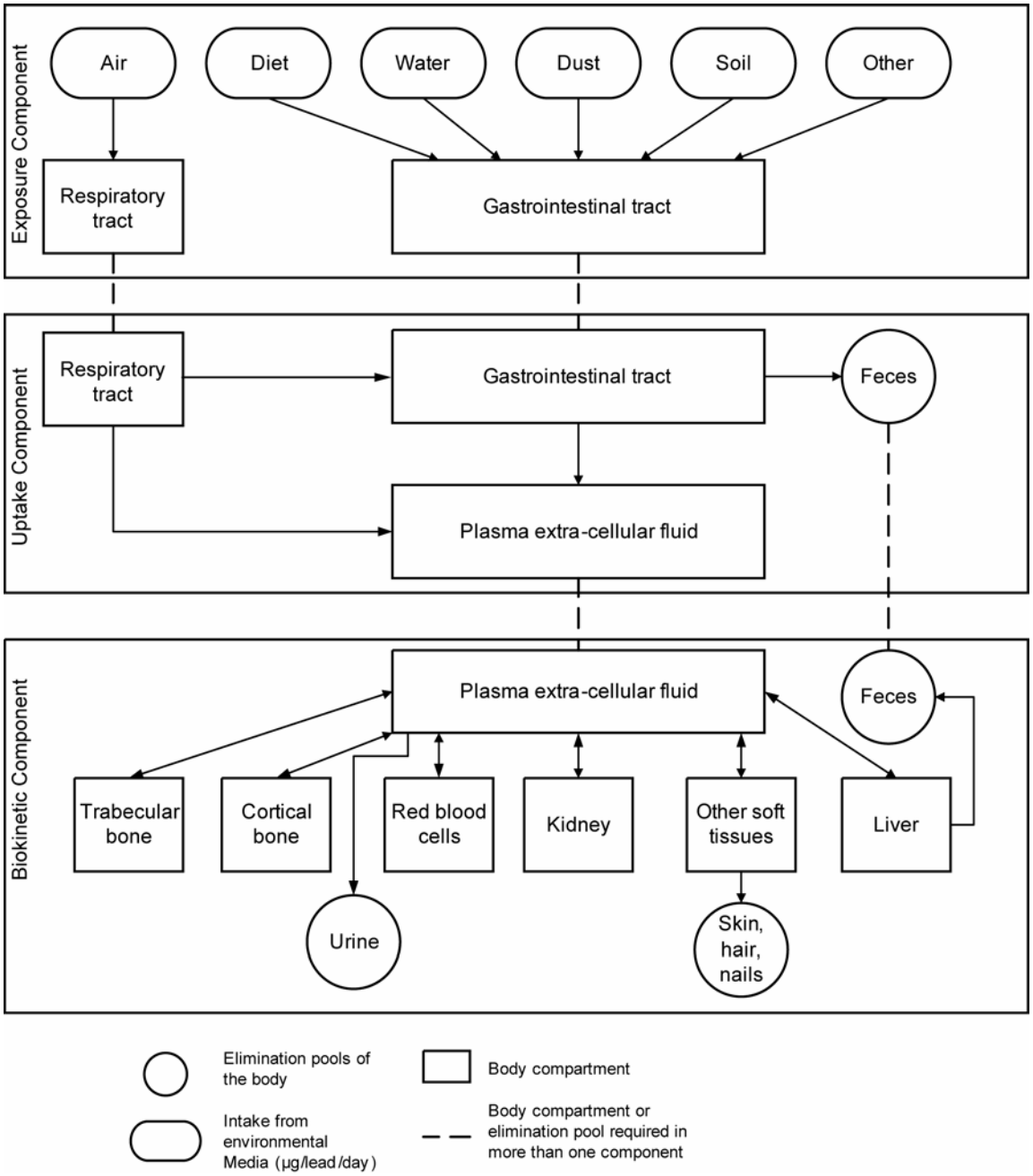

Figure 4-23. Structure of the integrated exposure uptake biokinetics model for lead in children (U.S. Environmental Protection Agency, 1994a,b; White et al., 1998).

was developed for predicting average quasi-steady state blood Pb concentrations corresponding to daily average exposures, averaged over periods ≥1 year.

The model has four major components or submodels:

- Exposure model, in which average daily intakes of Pb (µg/day, averaged over a 1 year time increment) are calculated for each inputted exposure concentration (or rates) of Pb in air, diet, dust, soil, and water;

- Uptake model, which converts environmental media-specific Pb intake rates calculated from the exposure model into a media-specific time-averaged rates of uptake (µg/day) of Pb to the central compartment (blood plasma);

- Biokinetic model, which simulates the transfer of absorbed Pb between blood and other body tissues, elimination of Pb from the body (via urine, feces, skin, hair, and nails), and predicts an average blood Pb concentration for the exposure time period of interest; and

- Blood Pb probability model, which simply applies a log-normal distribution (with specific geometric mean and geometric standard deviation parameters) to predict probabilities for the occurrence of a specified blood Pb concentration in a population of similarly exposed children.

Exposure Model. The exposure model simulates intake of Pb (µg/day) for exposures to Pb in air (µg/m^3), drinking water (µg/L), soil-derived dust (µg/g), and diet (µg/day). The temporal resolution of the exposure model is 1 year; exposure inputs are intended to represent annual averages for an age-year time step (e.g., ages 1, 2, 3...years). Exposure inputs that represent the average daily value for an age-year will yield corresponding daily average intakes for the same age-year. The spatial resolution of the exposure model was intended to be a child's residence (e.g., the home and yard). The model accepts inputs for media intake rates (e.g., air volume breathing rates, drinking water consumption rate, soil and dust ingestion rate). The air exposure pathway partitions exposure to outdoor air and indoor air; with age-dependent values for time spent outdoors and indoors (hours/day). Exposure to Pb in soil derived dust is also partitioned into outdoor and indoor contributions. The intakes from all ingested exposure media (diet, drinking water, soil-derived dust) are summed to calculate a total intake to the gastrointestinal tract, for estimating capacity-limited absorption (see description below of the Uptake Model).

Uptake Model. The uptake model simulates Pb absorption in the gastrointestinal tract as the sum of a capacity-limited (represented by a Michaelis-Menten type relationship) and unlimited processes (represented by a first-order, linear relationship). These two terms are intended to represent two different mechanisms of Pb absorption, an approach that is in accord with limited available data in humans and animals that suggest a capacity limitation for Pb

absorption (Mushak, 1991). One of the parameters for the capacity-limited absorption process (that represents that maximum rate of absorption) is age-dependent. The above representation gives rise to a decrease in the fractional absorption of ingested Pb as a function of total Pb intake as well as age. Absorption fractions are also medium-specific (Figure 4-24).

At 30 months of age, at low intakes (<200 μg/day), below the rates at which capacity-limitation has a significant impact on absorption, the fraction of ingested Pb in food or drinking water that is absorbed is 0.5 and decreases to 0.1 at high intake (5000 μg/day). For Pb ingested in soil or dust, fractional absorption is 0.3 at low intake (<200 μg/day) and decreases to 0.1 at high intake (5000 μg/day).

The uptake model has a default absorption rate of 32% (percent of inhaled Pb that is absorbed). This default absorption rate was intended to be an intermediate value between the absorption rate of 25% to 45% for young children living in non-point source areas and a rate of 42% for children living near point sources (U.S. Environmental Protection Agency, 1994b). In derivation of these absorption rates, it was assumed that Pb deposited in the alveolar region is completely absorbed, whereas, Pb deposited in the nasopharyngeal and tracheobronchial regions is transported to the gastrointestinal tract where ~40% absorption occurs. Furthermore, this default absorption rate was based on a respiratory particle deposition fraction for the particle size distribution to which children were assumed to be exposed. If sufficient information about a child's exposure is available, it is possible to calculate particle deposition fractions using publicly available particle dosimetry models (see Section 4.2.1). Using these new particle deposition fraction data, a recalculated absorption rate can be used in place of the default value.

Biokinetics Model. The biokinetics model includes a central compartment, plasma and extracellular fluid combined (plasma-ECF), six peripheral body compartments, and three elimination pathways. The temporal resolution of the biokinetics model is 1 month and, as discussed below, parameter values for bone-plasma-ECF exchanges were assigned with the objective of simulating the quasi-steady state condition of months, rather than short-term kinetics of days. The body compartments include kidney, liver, trabecular bone, cortical bone, and other soft tissue. The model simulates growth of the body and tissues, compartment volumes, and Pb masses and concentrations in each compartment. Blood Pb concentration at birth (neonatal) is assumed to be 0.85 of the maternal blood Pb (see Section 4.2.2. for discussion of observed

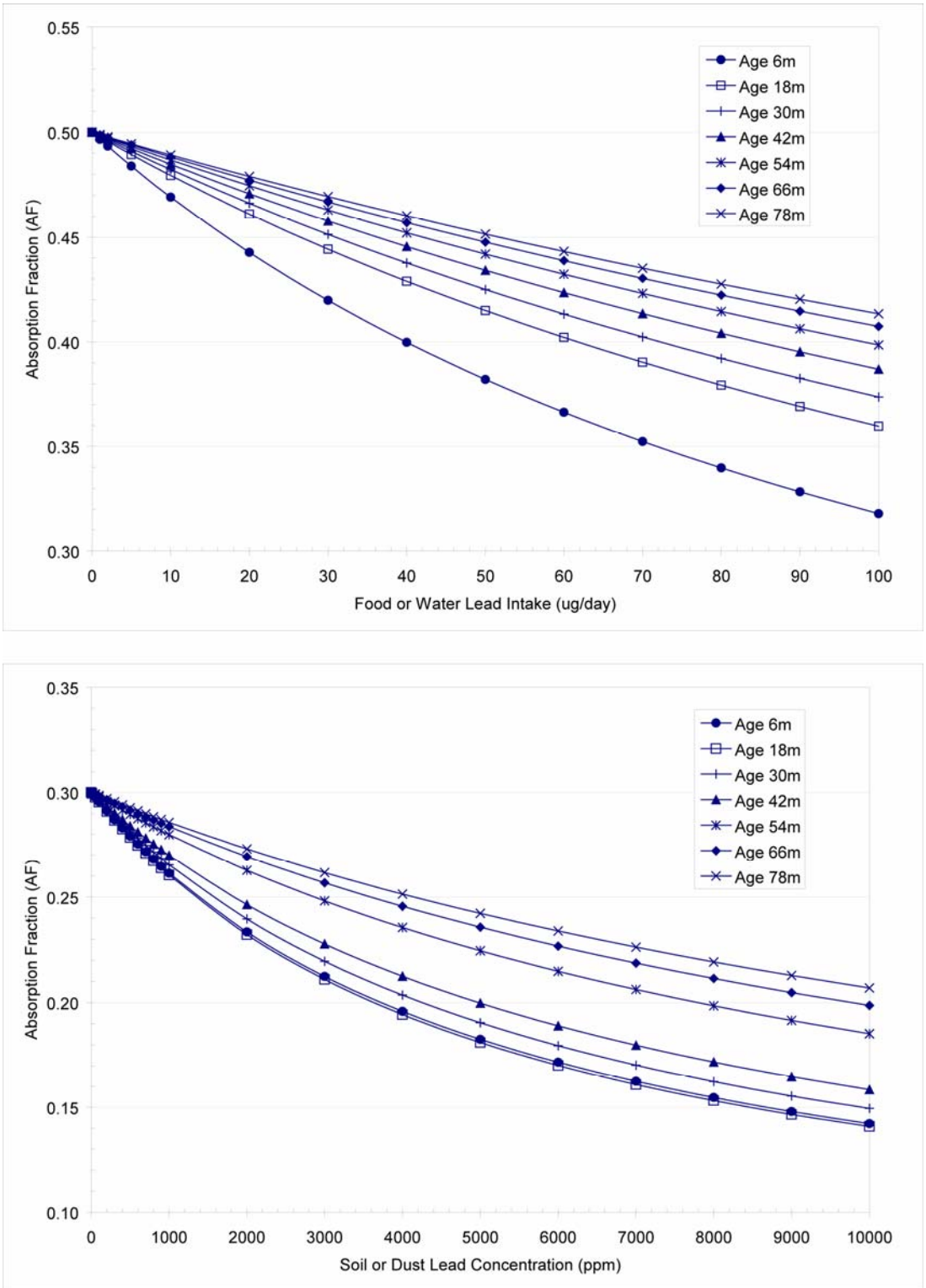

Figure 4-24. Age-dependency of absorption fraction for ingested lead in the IEUBK model for lead in children. Absorption fraction for food and water (top panel); soil and dust (bottom panel).

neonatal-maternal blood Pb concentration relationships). Neonatal Pb masses and concentrations are assigned to other compartments based on a weighted distribution of the neonatal blood Pb concentration. Exchanges between the central compartment and tissue compartments are simulated as first-order processes, which are parameterized with unidirectional, first-order rate coefficients. Rate coefficients are allometrically scaled as a power function of body weight ($BW^{0.33}$). Saturable uptake of Pb into erythrocytes is simulated, with a maximum erythrocyte Pb concentration of 120 µg/L. Excretory routes simulated include urine, from the central compartment; bile-feces, from the liver; and a lumped excretory pathway representing losses to skin, hair and nails, from the "other soft tissue" compartment.

Bone is simulated as a trabecular bone compartment (20% of bone volume) and a cortical bone compartment (80% of bone volume). Rate constants for transfer from plasma to the two bone compartments are assigned values that result in a 4:1 cortical Pb:trabecular Pb mass ratio within one biokinetic time step (one month). This is achieved by assigning the two bone compartments identical rate coefficients for transfer of Pb from bone to plasma-ECF (half-time 8.5 days, at age 2 years), and *faster* (cortical, half-time 0.0083 days) and *slower* transfer (trabecular, half-time 0.035 days) from the plasma-ECF (cortical:trabecular rate ratio is ~4:1). Note, this approach is different from previous and subsequent modeling approaches, in which cortical bone-to-plasma (or blood) transfer is assumed to occur slowly, relative to trabecular bone-to-plasma transfer (Marcus, 1985a; Bert et al., 1989; Leggett, 1993; O'Flaherty, 1993, 1995). For predictions of quasi-steady state conditions and the intended use of the IEUBK Model, the two general approaches can be expected to yield similar distributions of Pb between the cortical and trabecular bone compartments.

Blood Lead Probability Model. Inputs to the IEUBK model are exposure point estimates that are intended to represent time-averaged central tendency exposures. The output of the model is a central tendency estimate of blood Pb concentration for children who might experience the inputted average exposures. However, within a group of similarly exposed children, blood Pb concentrations would be expected to vary among children as a result of inter-individual variability in media intakes (e.g., daily average intakes of soil-derived dust, drinking water, or food), absorption, and biokinetics. The model simulates the combined impact of these sources of variability as a lognormal distribution of blood Pb concentration for which the geometric mean (GM) is given by the central tendency blood Pb concentration outputted from

the biokinetics model, and the geometric standard deviation (GSD) is an input parameter. The resulting lognormal distribution also provides the basis for predicting the probability of occurrence of given blood Pb concentration within a population of similarly exposed children:

$$P_{10} = \text{probability of exceeding a blood Pb concentration of 10 } \mu\text{g/dL} \qquad (4\text{-}14)$$

The model can be iterated for varying exposure concentrations (e.g., a series of increasing soil Pb concentration) to predict the media concentration that would be associated with a probability of 0.05 for the occurrence of a blood Pb concentration exceeding 10 µg/dL ($P_{10} = 0.05$).

4.4.5.2 Model Calibration and Evaluation

Evaluations of the IEUBK model have been carried out by comparison of model predictions of blood Pb concentrations in children with observations from epidemiologic studies of hazardous waste sites (Hogan et al., 1998; Bowers and Mattuck, 2001; TerraGraphics, Inc., 2000, 2001). Data characterizing residential Pb exposures and blood Pb concentrations in children living at four Superfund National Priorities List (NPL) sites were collected in a study designed by the Agency for Toxic Substances and Disease Registry (ATSDR) and EPA. The residential exposure data were used as inputs to the IEUBK model and predicted blood Pb concentration distributions were compared to the observed distributions in children living at the same residences. The IEUBK model predictions of geometric mean blood Pb concentrations for children whose exposures were predominantly from their residence (i.e., no more than 10 hours/week away from home) were within 0.7 µg/dL of the observed geometric mean at each site (Table 4-15). The prediction of the percentage of children expected to have blood Pb concentrations exceeding 10 µg/dL were within 4% of the observed percentage at each site (Table 4-16). A similar type of empirical comparison was conducted by Bowers and Mattuck (2001) based on data from 4 mining and/or smelting sites. The results from both studies (Hogan et al., 1998 and Bowers and Mattuck, 2001) are shown in Figure 4-25. TerraGraphics, Inc. (2000) reported predicted and observed blood Pb concentrations in 2-year old children who resided at the Bunker Hill, ID site during the period 1988-1998. Comparisons of observed and

Table 4-15. Comparison of Observed and Predicted Geometric Mean Blood Lead for Three Community Blood Lead Studies

Dataset	N	Observed Blood Lead (μg/dL)		Model Predictions (μg/dL)	
		GM	95% CI	GM	95% CI
Galena, KA Jasper Co, MI[a]	111	5.2	4.5-5.9	4.6	4.0-5.3
Madison Co, IL[a]	333	5.9	5.5-6.4	5.9	5.4-6.3
Palmerton, PA[b]	34	6.8	5.6-8.2	7.5	6.6-8.6

CI = confidence interval; GM = geometric means

[a]Children away from home ≤10 hours/week
[b]Children away from home ≤20 hours/week

Table 4-16. Comparison of Observed and Predicted Probability of Exceeding a Blood Lead Concentration of 10 μg/dL Lead for Three Community Blood Lead Studies

Dataset	N	Observed Blood Lead (μg/dL)		Model Predictions (μg/dL)	
		Percent	95% CI	Percent	95% CI
Galena, KA Jasper Co, MI[a]	111	20	13-27	18	11-25
Madison Co, IL[a]	333	19	15-23	23	19-28
Palmerton, PA[b]	34	29	14-44	31	16-47

CI, confidence interval

[a]Children away from home ≤10 hours/week
[b]Children away from home ≤20 hours/week

predicted GM blood Pb concentration and P_{10} are shown in Figure 4-26. Empirical comparisons have shown that agreement or disparity between IEUBK model predictions and observed blood Pb concentrations at specific locations is influenced by numerous factors, including (a) the extent to which the exposure and blood Pb measurements are adequately matched and (b) site-specific factors (e.g., soil characteristics, behavior patterns, bioavailability) that may affect Pb intake or

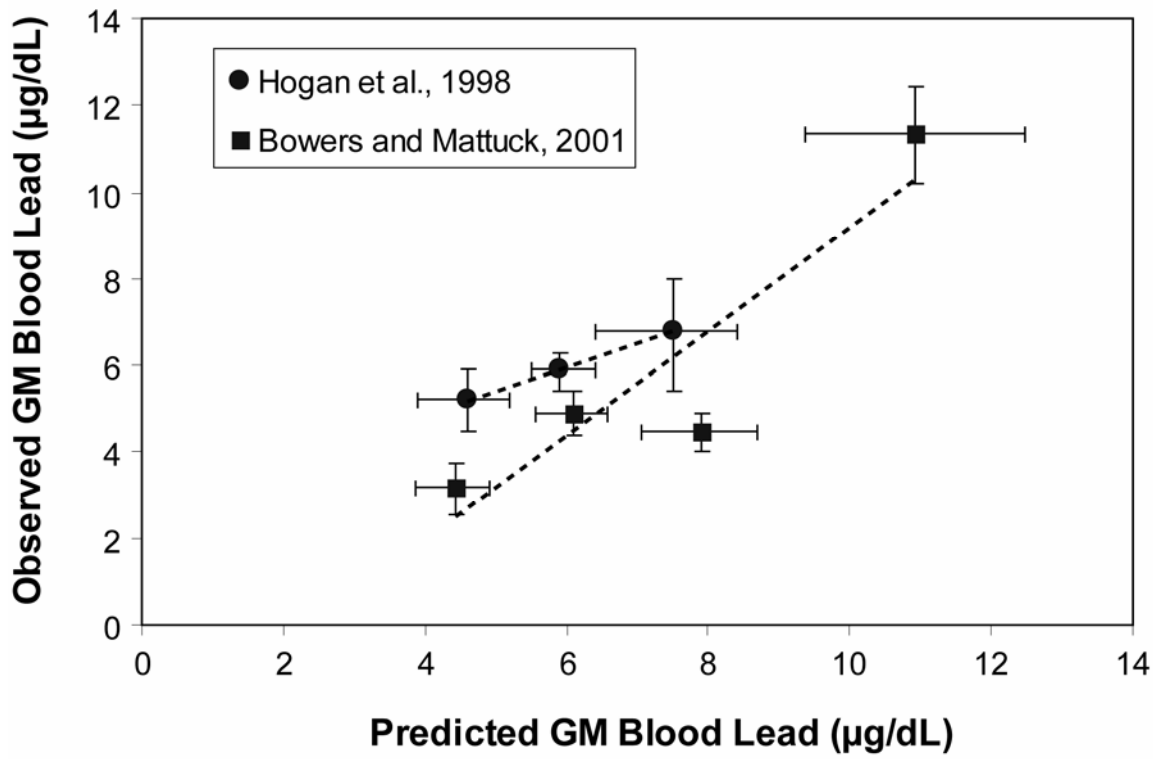

Figure 4-25. Comparisons of IEUBK model predictions and observed blood lead concentrations. Data from Hogan et al. (1998) include observations from 3 sites (left to right): Galena, KS and Jasper Co, MO, combined, N=111; Madison Co, IL, N=333; and Palmerton, PA, N=34. Data from Bowers and Mattuck (2001) include observations from 4 sites (left to right): Sandy, UT (reported as Sandy, UT-2), N=36; Midvale, UT, N=151; Palmerton, PA, N=110; and Cincinnati, OH, N=95. Bars are 95% confidence limits; dashed lines represent simple linear regression models based on means (not individual child data) from each study (Hogan et al., r^2>0.99; Bowers and Mattuck, r^2=0.84; these r^2 are the fit to the group means and exceed the r^2 that would be expected for the complete datasets).

uptake in children. Error in measurement of exposure, by itself, can be expected to attenuate the predicted slope of the relationship between exposure and blood Pb concentration (Carrol and Galindo, 1998; Marcus and Elias, 1998). In the absence of a suitable dataset of paired environmental Pb and blood Pb measurements at a given site, it is not possible to ascertain the degree to which the model predictions will represent the exposure-blood Pb concentration relationships at that site (Bowers and Mattuck, 2001).

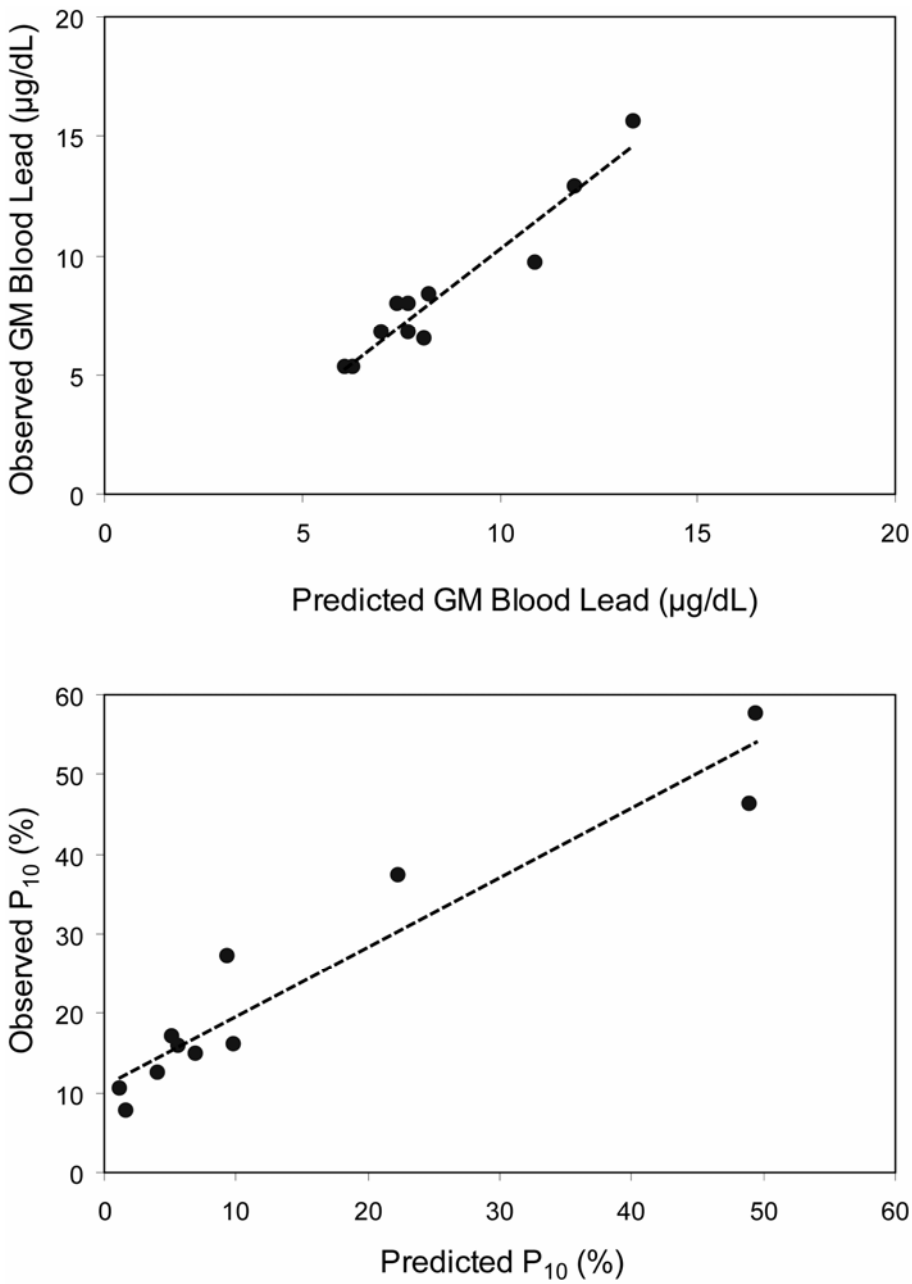

Figure 4-26. Comparison of IEUBK model predictions and observed blood lead concentrations. Data are annual (1988-1998, N = 23 to 57 each year) site-wide assessments of 2-year old children who resided at the Bunker Hill site in northern Idaho. Soil and dust lead bioavailability was assumed to be 18%. Relative contributions of soil and house dust to total soil/dust ingestion were assumed to be 42% interior dust; 27% community soil; 10% neighborhood soil (within 100-ft radius of residence); and 12% yard soil. Dashed lines represent simple linear regression models applied to the geometric means (r^2 = 0.92*) or P10s (r^2 = 0.91*). *Note that this r^2 is for the fit of the model to the GM and does not consider the additional variability of the complete data sets.

4.4.5.3 Model Applications

The U.S. EPA has recommended that the IEUBK model be used to assess health risks to children exposed to contaminated soils at hazardous waste sites (U.S. Environmental Protection Agency, 1994c, 1998).

Biomarkers Simulated. The IEUBK model computes masses of Pb in bone and various soft tissues, and excretion of Pb, which are used in the computation of blood Pb concentration. However, the model was not developed for the purpose of predicting Pb masses in these tissues or excreta. Blood Pb concentration is the only Pb biomarker output that is accessible to the user.

Exposure Inputs. The IEUBK model was developed to predict the probability of elevated blood Pb concentrations in children exposed to user-specified annual average exposures to Pb in air, food, drinking water, soil, and dust. As noted above, the exposure model has an age-year time step (the smallest time interval for a single exposure event) and, therefore, is more suited to applications in which long-term (i.e., ≥ 1 year) average exposures and quasi-steady state blood Pb concentrations are to be simulated. Intermittent exposures occur for brief periods of time (e.g., a weekend at the beach), or in cases where significant seasonal variations are different from the typical residential or occupational exposure. Intermittent exposures can be simulated as time-weighted average exposures (U.S. Environmental Protection Agency, 2003a). Shorter-term dynamics of blood Pb concentration, that may result from exposures that are highly variable on time scales of days or weeks, will not be captured with this approach (Lorenzana et al., 2005; Khoury and Diamond, 2003).

Modeling Variability and Uncertainty. As noted above, the IEUBK model uses a lognormal probability model to simulate inter-individual variability in blood Pb concentrations attributable to variability in media intakes, absorption, and biokinetics. The model provides a generic default value of 1.6 for the geometric standard deviation (GSD_i) of blood Pb concentrations. This value, which can be altered by the user, was derived from an analysis of exposure (soil Pb)-stratified variability in blood Pb concentrations in various cohorts of children (U.S. Environmental Protection Agency, 1994a; White et al., 1998). Griffin et al. (1999b) also explores various statistical methods for estimating an appropriate GSD_i (regression, box modeling, structural equation modeling).

A Monte Carlo approach has been used to simulate and propagate variability and uncertainty in exposure and absorption through IEUBK model simulation of blood Pb

concentrations (Goodrum et al., 1996). This extension of the model provides an alternative to the generic blood Pb probability approach for incorporating explicit estimates of variability (and uncertainty in variability) in exposure and absorption into predictions of an expected probability distribution of blood Pb concentrations. A quantitative uncertainty analysis of IEUBK model-based estimates of the P_{10} for a smelter site in Utah revealed that parameters specifying soil ingestion rate were a dominant contributor to uncertainty in the P_{10}; however, the contribution of soil ingestion uncertainty, relative to uncertainty in other model parameters (i.e., mean soil Pb concentration, absorption fraction) varied across individual locations (Initial Study Zones) at the site (Griffin et al., 1999a).

4.4.5.4 Implementation Code

The IEUBK model was initially released to the public in 1994 as a compiled DOS-based C program (IEUBK v99d). This version was subjected to an independent code validation and verification study which verified that the code accurately implement the model (Mickle, 1998; Zaragoza and Hogan, 1998). A 32-bit C++ (IEUBKwin32) version of the model is available for download from an EPA website (http://www.epa.gov/superfund/programs/Pb/ieubk.htm). The IEUBKwin32 program outputs blood Pb concentrations and probabilities of exceeding a given blood Pb concentration. Levels of Pb in other tissue compartments are computed but are not accessible to the user.

4.4.6 Leggett Model

4.4.6.1 Model Structure

The Leggett model was developed from a biokinetic model originally developed for the International Commission on Radiological Protection (ICRP) for calculating radiation doses from environmentally important bone-seeking radionuclides, including radioisotopes of Pb (Leggett, 1985, 1992a,b). The model has been used to develop cancer risk coefficients for internal radiation exposures to Pb and other alkaline earth elements that have biokinetics similar to those of calcium (ICRP, 1993; U.S. Environmental Protection Agency, 1998a). The model includes a central exchange compartment, 15 peripheral body compartments, and 3 elimination pools (Figure 4-27). The central exchange compartment is the diffusible pool of Pb in plasma. The model simulates a bound pool in plasma (i.e., Pb bound to plasma proteins); that has an

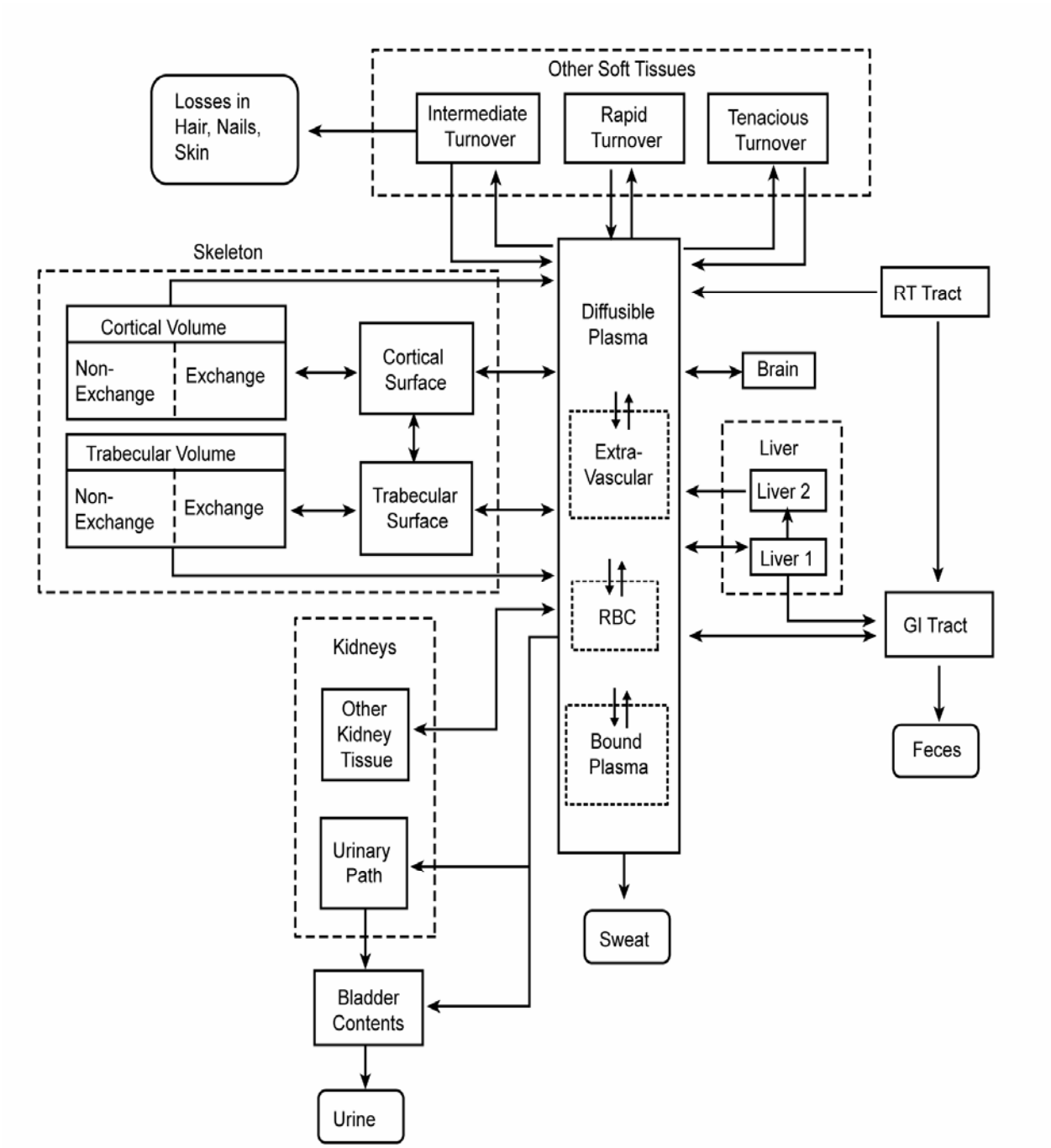

Figure 4-27. Structure of the Leggett Lead Biokinetic Model (Leggett, 1993). The central exchange compartment is *diffusible* plasma. Bone is represented as having surface (which rapidly exchanges with plasma) and volume compartments; the latter stimulates slow exchange with the surface and slow return of lead to the plasma from bone resorption.

equilibrium ratio (bound:free) of ~5. Transport of Pb from plasma to tissues is assumed to follow first-order kinetics. The temporal resolution of the model is 1 day. Transfer rate constants vary with age and blood Pb concentration. The latter adjustment accounts for the limited uptake of plasma Pb into red blood cells and the resulting shift in distribution of Pb from plasma-ECF to other tissues. Above a nonlinear threshold concentration in red blood cells (assumed to be 60 µg/dL), the rate constant for transfer to red blood cells declines and constants to all other tissues increase proportionally (Leggett, 1993). This replicates the nonlinear relationship between plasma and red blood observed in humans (Smith et al., 2002; Manton et al., 2001; Bergdahl et al., 1997a, 1998, 1999). The model simulates blood volume as an age-dependent function, which allows simulation of plasma and blood Pb concentrations. However, volumes of other tissues are not simulated; therefore, only Pb masses in these tissues, and not concentrations are simulated.

First-order transfer coefficients (day-1) between compartments were developed for six age groups, and intermediate age-specific values are obtained by linear interpolation (Leggett, 1993). The total transfer rate from diffusible plasma to all destinations (TPALL) combined is assumed to be 2000 day-1, based on isotope tracer studies in humans receiving Pb via injection or inhalation. Values for transfer coefficients from plasma to tissues and tissue compartments are based on measured deposition fractions (DF) or instantaneous fractional outflows of Pb between tissues compartments (Leggett, 1993), where the transfer coefficient to a specific tissue or compartment (TP_i) is given by:

$$TP_i = DF_i \cdot TPALL \tag{4-15}$$

This approach establishes mass balance with respect to the transfer rates from plasma:

$$\sum TP_i = TPALL \tag{4-16}$$

The model simulates both rapid exchange of Pb with plasma via bone surface and slow loss by bone resorption. Cortical bone volume (80% of bone volume) and trabecular bone volume (20% of bone volume) are simulated as bone surface compartments, which rapidly exchanges with Pb in plasma, and bone volume, within which are *exchangeable* and *nonexchangeable* pools. Lead enters the exchangeable pool of bone volume via the bone surface

and can return to the bone surface, or move to the nonexchangeable pool, from where it can return to the plasma only when bone is resorbed. Transfers from plasma to bone surface, return from bone surface to plasma, and bone surface to exchangeable bone volume are assumed to be relatively fast processes (adult $t_{1/2}$ = 3.85, 1.4, and 1.4 days, respectively). Return of Pb from the exchangeable bone volume is slower (adult $t_{1/2}$ = 30 days); however, the dominant transfer process determining long-term accrual of bone Pb burden are slow rate coefficients for transfer of Pb from the nonexchangeable pools of trabecular and cortical bone to plasma (adult $t_{1/2}$ = 3.8 and 23 years, respectively). Bone transfer coefficients vary with age (faster in children) to reflect the age-dependence of bone turnover. The slow, nonexchangeable, bone volume compartment is much more labile in infants and children than in adults (e.g., cortical $t_{1/2}$ = 68 days at birth and 1,354 days at age 15 years; trabecular $t_{1/2}$ = 68 days at birth and 725 days at age 15 years). Other physiological states (such as pregnancy and menopause) that affect bone turnover and, therefore, bone Pb kinetics are not simulated, although such states could conceivably be accommodated with adjustments to tissue (e.g., bone) transfer coefficients.

The liver is simulated as two compartments; one compartment has a relatively short removal half-life for transfers to plasma and to the small intestine by biliary secretion (adult $t_{1/2}$ = 10 days); a second compartment simulates a more gradual transfer to plasma of ~10% of Pb uptake in liver (adult $t_{1/2}$ = 365 days). The kidney is simulated as two compartments, one that exchanges slowly with blood plasma and accounts for Pb accumulation in kidney tissue (adult $t_{1/2}$ = 365 days) and a second compartment that receives Pb from blood plasma and rapidly transfers Pb to urine (adult $t_{1/2}$ = 5 days), with essentially no accumulation (urinary pathway). Other soft tissues are simulated as three compartments representing rapid, intermediate, and slow turnover rates, without specific physiologic correlates (adult $t_{1/2}$ = 0.3, 100, and 1824 days, respectively). Other excretory pathways (hair, nails, and skin) are represented as a lumped pathway from the intermediate turnover rate of the soft tissue compartment.

The Leggett model simulates Pb intakes from inhalation, ingestion, or intravenous injection. The latter was included to accommodate model evaluations based on intravenous injection studies in humans and animal models. The respiratory tract is simulated as four compartments into which inhaled Pb is deposited and absorbed with half-times of 1, 3, 10, and 48 hours. Five percent of the inhaled Pb is assumed to be transferred to the GI tract. These parameter values reflect the data on which the model was based, which were derived from

studies in which human subjects inhaled submicron Pb-bearing particles (Morrow et al., 1980; Chamberlain et al., 1978; Wells et al., 1977; Hursh and Mercer, 1970; Hursh et al., 1969).

These assumptions would not necessarily apply for exposures to larger airborne particles (see Sections 2.3.1 for a discussion of atmospheric transport of Pb particles). Absorption of ingested Pb is simulated as an age-dependent fraction of the ingestion rate, declining from 0.45 at birth to 0.3 at age 1 year (to age 15 years), and to 0.15 after age 25 years (Figure 4-28).

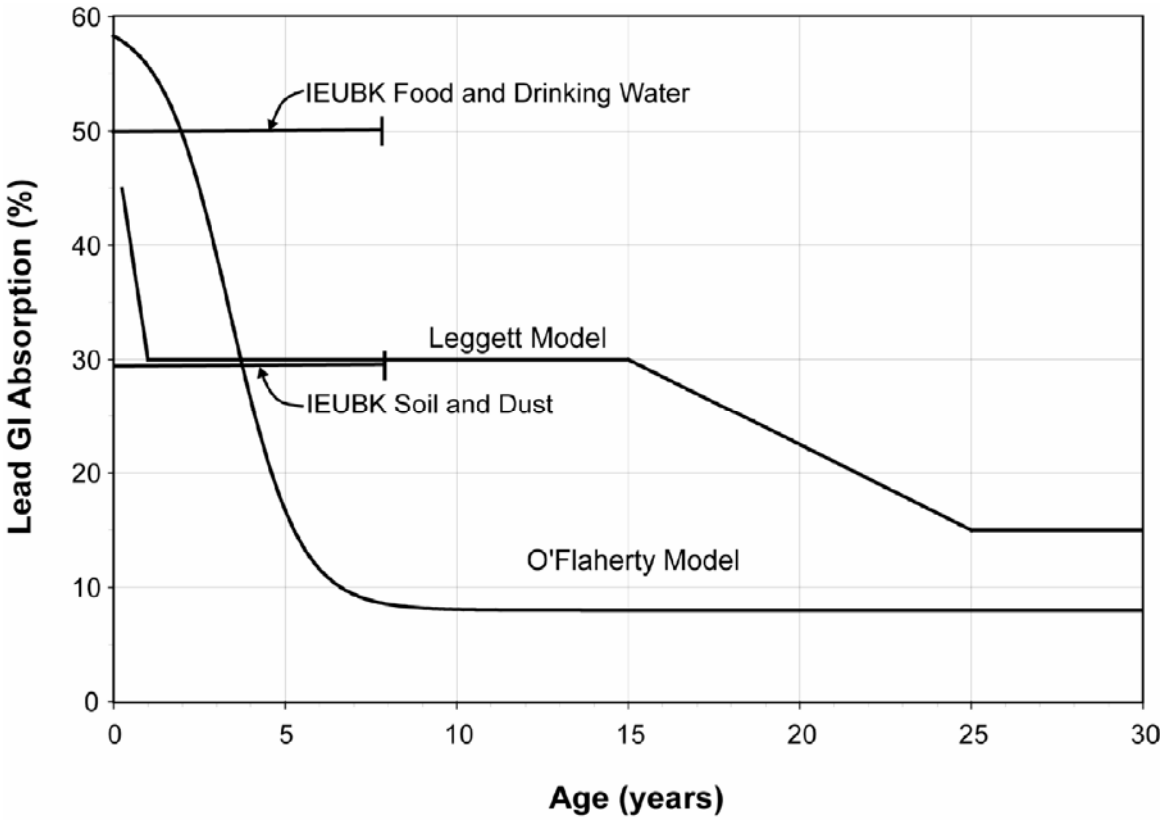

Figure 4-28. Age-dependency of absorption fraction for ingested lead in the Leggett and O'Flaherty models. The IEUBK model projects absorption only through age seven (84 mo). At intakes below those which approach the limit on "active" absorption of lead, absorption is constant with age, with default valves of 50% for diet and drinking water, 30% for soil and dust. Fractional absorption via the active pathway decreases with age and lead intake (see Figure 4-23).

4.4.6.2 Model Calibration and Evaluation

Leggett (1993) and Pounds and Leggett (1998) describe various qualitative empirical comparisons of model predictions against observations made on adults (e.g., Skerfving et al., 1985; Campbell et al., 1984; Manton and Cook, 1984; Barry, 1981; DeSilva, 1981; Chamberlain et al., 1978; Rabinowitz et al., 1976; Barry, 1975; Griffin et al., 1975; Gross et al., 1975; Hursh and Mercer, 1970; Booker et al., 1969; Hursh et al., 1969; Schroeder and Tipton, 1968). Age-specific changes in parameter values that specify the biokinetics of Pb in children were assigned values that resulted in agreement between predicted age-specific Pb distribution (fraction of body burden) in blood, bone, brain, kidney, liver, and other tissues, and reported postmortem values (Schroeder and Tipton, 1968; Barry, 1975, Gross et al. 1975; Barry, 1981). Comparisons of model predictions to observed relationships between plasma and red blood cell Pb levels are reported in U.S. Environmental Protection Agency (2003b).

4.4.6.3 Model Applications

Biomarkers Simulated. The Leggett model simulates the concentrations of Pb in blood and plasma, masses of Pb in bone and various soft tissues, and excretion of Pb in urine that correspond to lifetime exposures (in terms of daily Pb intakes).

Exposure Inputs. The model does not contain a detailed exposure module (although it can be linked to an exposure model); Pb exposure estimates are incorporated into the simulations as age-specific point estimates of daily intake (µg/day) from ingestion, inhalation, or injection. The model operates with a Pb intake time step of 1 day, which allows simulation of rapidly changing (i.e., daily) intermittent exposures (Lorenzana et al., 2005; Khoury and Diamond, 2003). Assumptions of blood Pb concentrations at birth can also be introduced into the simulations, from which levels in other tissue in the first time step after birth are calculated.

Dose reconstruction is possible with this model, since intakes, and corresponding tissue Pb burdens accrued at any period in the lifetime, prior to an exposure event of interest, can be simulated. Pounds and Leggett (1998) illustrate this in a study of a childhood Pb poisoning case, in which the exposure is followed by chelation. Chelation was simulated as a short-duration increase in the plasma Pb deposition fraction to urine, with corresponding proportional decreases in deposition fractions to other tissues.

4.4.6.4 Implementation Code

The Leggett model was initially developed as a Fortran code, which can be run, without compiling, from various platforms, including DOS and Windows (see Pounds and Leggett, 1998 for a description). A version compiled in Advanced Continuous Simulation Language (ACSL) has also been reported (Lorenzana et al., 2005). Confirmation of the Leggett model code was carried out by a panel of experts (ICRP, 1989, 1993).

4.4.7 O'Flaherty Model

4.4.7.1 Model Structure

The O'Flaherty model simulates Pb exposure, uptake, and disposition in humans, from birth through adulthood (O'Flaherty, 1993, 1995, 2000). Figure 4-29 shows a conceptualized representation of the model. Important novel features of the O'Flaherty model are the simulation of growth, bone formation, and resorption. A growth curve is simulated with a logistic expression relating body weight to age in males or females. The full expression relating weight to age has five parameters (constants), so that it can readily be adapted to fit a range of standardized growth curves for males and females. Tissue growth and volumes are linked to body weight; this provides explicit modeling of Pb concentrations in all tissues simulated. Other physiologic functions (e.g., bone formation) are linked to body weight, age, or to both. The model can be implemented with a temporal resolution of 1 day; however, as originally configured, the rate parameters are expressed in time units of years.

Rates of bone formation and resorption are simulated as age-dependent functions (Figure 4-30). Uptake and release of Pb from trabecular bone and metabolically active cortical bone are functions of bone formation and resorption rates, respectively; this establishes the age-dependence to the Pb kinetics in and out of bone. Lead exchange between blood plasma and bone is simulated as parallel processes occurring in cortical (80% of bone volume) and trabecular bone (20% of bone volume). The model simulates an age-related transition from immature bone, for which bone turnover (formation and resorption) rates are relatively high, to mature bone, for which turnover is relatively slow. Changes in bone mineral turnover associated with aging and senescence (e.g., postmenopausal osteoporosis) can be simulated by introducing an age-dependent increase rate of bone resorption (O'Flaherty, 2000). Metabolically active regions of bone, in which Pb uptake and loss is dominated by bone formation and loss, a region of slow

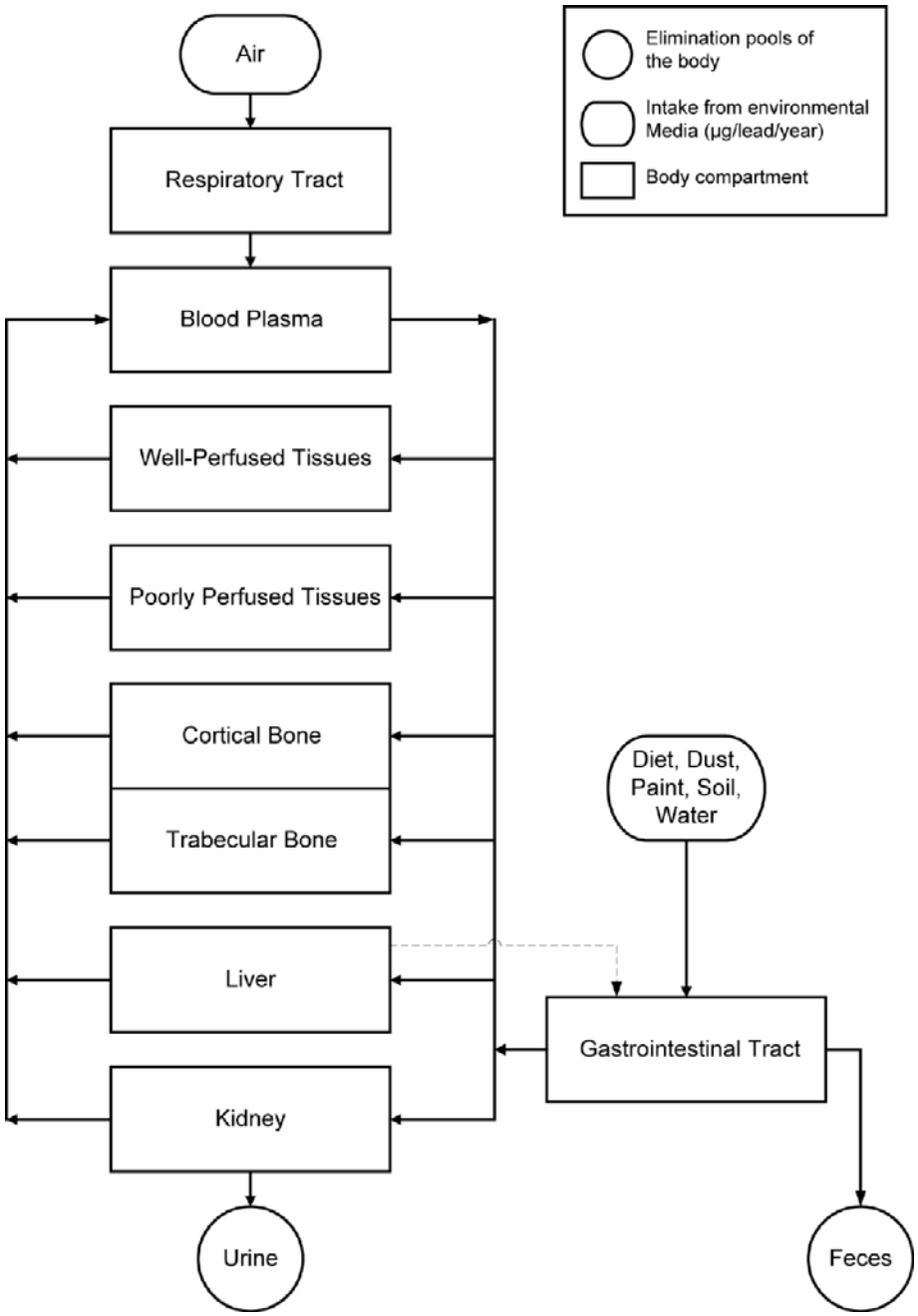

Figure 4-29. Structure of the O'Flaherty Lead Exposure Biokinetics Model (O'Flaherty, 1993, 1995, 2000). The central exchanges compartment is *diffusible* plasma. Lead distribution is represented by flows from blood plasma to liver, kidney, richly-perfused tissues, poorly-perfused tissues, and cortical and trabecular bone. The model simulates tissue growth with age, including growth and resorption of bone mineral.

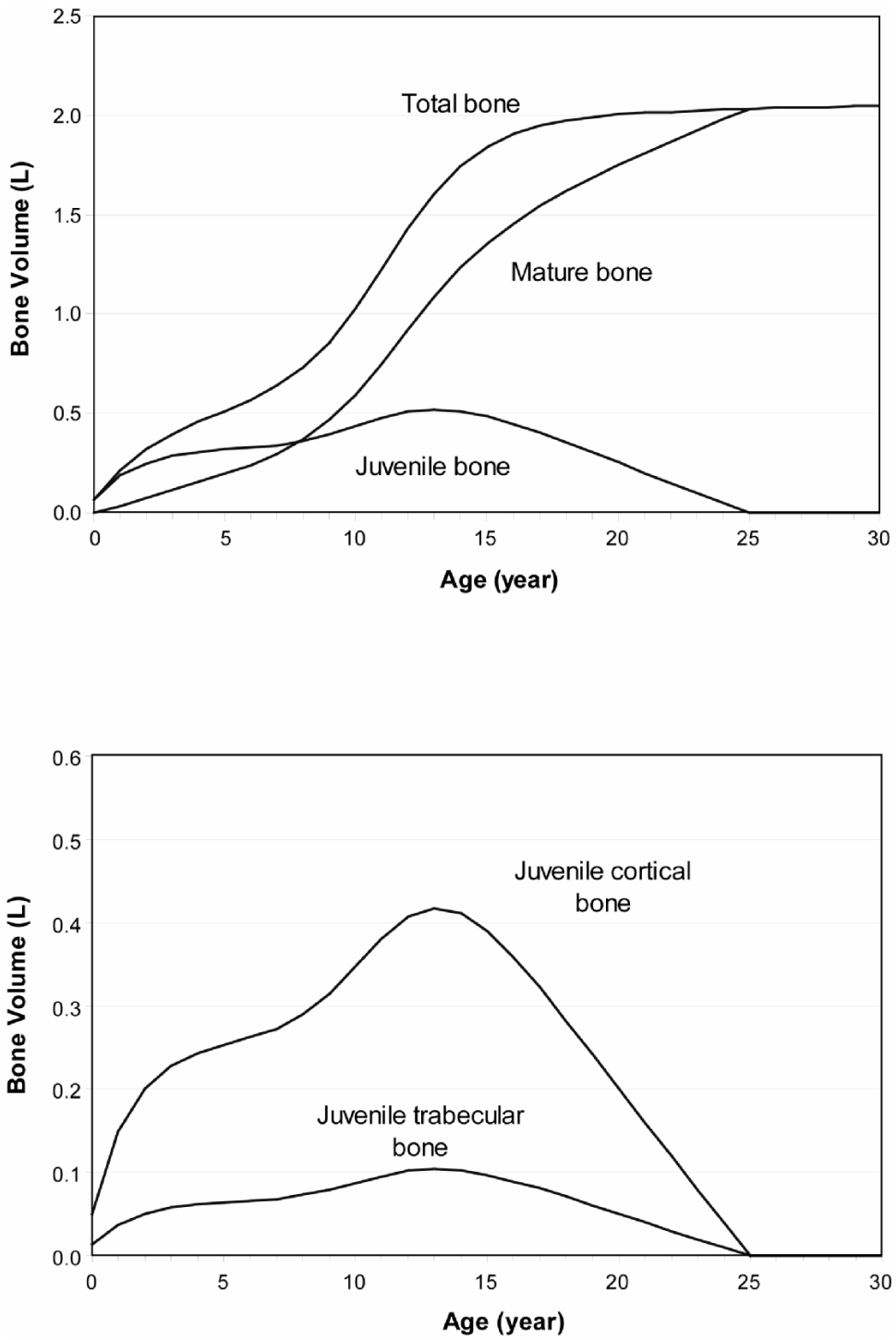

Figure 4-30. Bone growth as simulated by the O'Flaherty Lead Exposure Biokinetics Model (O'Flaherty, 1993, 1995, 2000). The model simulates an age-related transition from juvenile bone, in which bone turn-over (formation and resorption) rates are relatively high, to mature bone, in which turn-over is relatively slow. Cortical bone comprises ~80% of total bone volume.

kinetics in mature cortical bone is also simulated, in which Pb uptake and release to blood occur by heteroionic exchange with other minerals (e.g., calcium). Heteroionic exchange is simulated as a radial diffusion in bone volume of the osteon. All three processes are linked to body weight or the rate of change of weight with age. This approach allows for explicit simulation of the effects of bone formation (e.g., growth) and loss, changes in bone volume, and bone maturation on Pb uptake and release from bone. Exchanges of Pb between blood plasma and soft tissues (e.g., kidney and liver) are represented as flow-limited processes. The model simulates saturable binding of Pb in erythrocytes (maximum capacity is 2.7 mg Pb/L cell volume); this replicates the curvilinear relationship between plasma and erythrocyte Pb concentrations observed in humans (Smith et al., 2002; Manton et al., 2001; Bergdahl et al., 1997a, 1998, 1999). Excretory routes include kidney to urine and liver to bile. Total excretion (clearance from plasma attributable to bile and urine) is simulated as a function of age-dependent glomerular filtration rate. Biliary and urinary excretory rates are proportioned as 70 and 30% of the total plasma clearance, respectively.

The O'Flaherty model simulates Pb intake from inhalation and ingestion. Inhalation rates are age-dependent. Absorption of inhaled Pb is simulated as a fraction (0.5) of the amount inhaled and is independent of age. Gastrointestinal absorption of Pb in diet and drinking water is simulated as an age-dependent fraction, declining from 0.58 of the ingestion rate at birth to 0.08 after age 8 years (Figure 4-28). These values can be factored to account for relative bioavailability when applied to absorption of Pb ingested in dust or soil.

4.4.7.2 Model Calibration and Evaluation

The O'Flaherty model was initially calibrated to predict blood, bone, and tissue Pb concentrations in rats (O'Flaherty, 1991a,b,c) and was later modified to reflect anatomical and physiological characteristics in children (O'Flaherty, 1995), adults (O'Flaherty, 1993), and Cynomolgus monkeys (*M. fasicularis*) (O'Flaherty et al., 1998). Model parameters were modified to correspond with available information on species- and age-specific anatomy and physiological processes. Empirical comparisons (largely qualitative) of model predictions against observations made in adults (e.g., Van De Vyver et al., 1988; Kehoe, 1987; Marcus, 1985c; Manton and Malloy, 1983; Sherlock et al., 1982; DeSilva, 1981; Moore et al., 1977; Cools et al., 1976; Rabinowitz et al., 1976; Azar et al., 1975) are provided in O'Flaherty (1993);

and comparisons against observations made in children (e.g., Sherlock and Quinn, 1986; Bornschein et al., 1985b; Chisolm et al., 1985; Lacey et al., 1985) and adults are described in O'Flaherty (1995, 1998, 2000).

4.4.7.3 Model Applications

Biomarkers Simulated. The O'Flaherty model simulates Pb concentrations in blood and plasma, bone, and various soft tissues, and excretion of Pb in urine that correspond to lifetime exposures (in terms of daily Pb intakes). Lead in feces is a mixture of unknown proportions of unabsorbed Pb in food, drinking water, ingested dust, a small amount of inhaled Pb entering the GI tract by the mucociliary clearance from the respiratory tract, and a small amount of absorbed Pb eliminated with the red blood cells passing along the bile duct to the GI tract. In this respect, Pb in feces represents a poorly defined measure of Pb exposure.

Lead in perspiration represents Pb in extracellular plasma, but the concentration is low and difficult to measure in a small volume (1 drop ≅ 0.05 mL) and is potentially contaminated with Pb in dust on the skin surface.

The model predicts blood Pb concentrations for a broad age range (infants to adults), which allows for simulated dose reconstruction, since intakes and corresponding tissue Pb burdens accrued at any period in the lifetime, prior to an exposure event of interest can be simulated. Physiological states (such as pregnancy and menopause) that affect bone turnover and, therefore, bone Pb kinetics are not simulated, although such states could be accommodated with adjustments to the physiological bone formation and resorption rates.

Exposure Inputs. The O'Flaherty model simulates Pb intake by inhalation and ingestion. The model simulates ingestion exposures from infant formula, soil, dust, and drinking water. Rates of soil and dust ingestion are age-dependent, increasing to ~130 mg/day at age 2 years and declining to <1 mg/day after age 10 years. However, the ACSL implementation code allows constructions of simulations with an exposure time step as small as 1 day, which would allow simulation of rapidly changing intermittent exposures (e.g., an acute exposure event).

Modeling Variability and Uncertainty. The O'Flaherty model, as described in O'Flaherty (1993, 1995), utilizes point estimates for parameter values and yields point estimates as output; however, a subsequent elaboration of the model has been reported that utilized a Monte Carlo approach to simulate variability in exposure, absorption, and erythrocyte Pb binding capacity

(Beck et al., 2001). This approach could be used to predict the probability that children exposed to Pb in environmental media will have blood Pb concentrations exceeding a health-based level of concern (e.g., 10 µg/dL).

4.4.7.4 Implementation Code

The O'Flaherty model was developed in ACSL and published in O'Flaherty (2000). A compiled C program has also been developed (personal communication, E. O'Flaherty). The extent to which code verification and validation studies have been conducted for the O'Flaherty model is unclear at this time. However, analogs of certain components of the O'Flaherty model (e.g., parameters related to bone growth) have been incorporated into the EPA All Ages Lead Model (AALM) (see Section 4.4.7) as a potential option for evaluation.

4.4.8 EPA All Ages Lead Model

4.4.8.1 Model Structure

The EPA All Ages Lead Model (AALM), currently under development, simulates lifetime Pb exposures and biokinetics in humans (Figure 4-31). The model is expected to simulate exposure and biokinetics of Pb from birth to age 90 years and should also incorporate, at some near-future time, a pregnancy module that simulates transplacental transfer of Pb from the mother to the fetus.

Exposure Module. The exposure component of the AALM incorporates and extends the exposure component of the IEUBK model. The AALM exposure model defines an individual in terms of age, sex, date of birth, and activity pattern profile. The age specification establishes up to nine age ranges (e.g., infant, child, adolescent, adult, etc.) for which various exposure (and biokinetic) parameter values can be applied. This provides a means for varying parameter values with age. The sex specification links the modeled individual to the appropriate growth algorithm (O'Flaherty 1993, 1995), and the date specification links the individual to historical exposure levels (e.g., air, diet) for the selected age range. The activity pattern specification sets the relative amount of time the individual spends in various exposure settings (e.g., residential, school, recreational, occupational) for which exposure concentrations can be specified.

The diet exposure module allows input values (current or historical) for Pb levels (µg/g) in market basket fruits, vegetables, meat and fish; recreational- or subsistence-harvested fish and

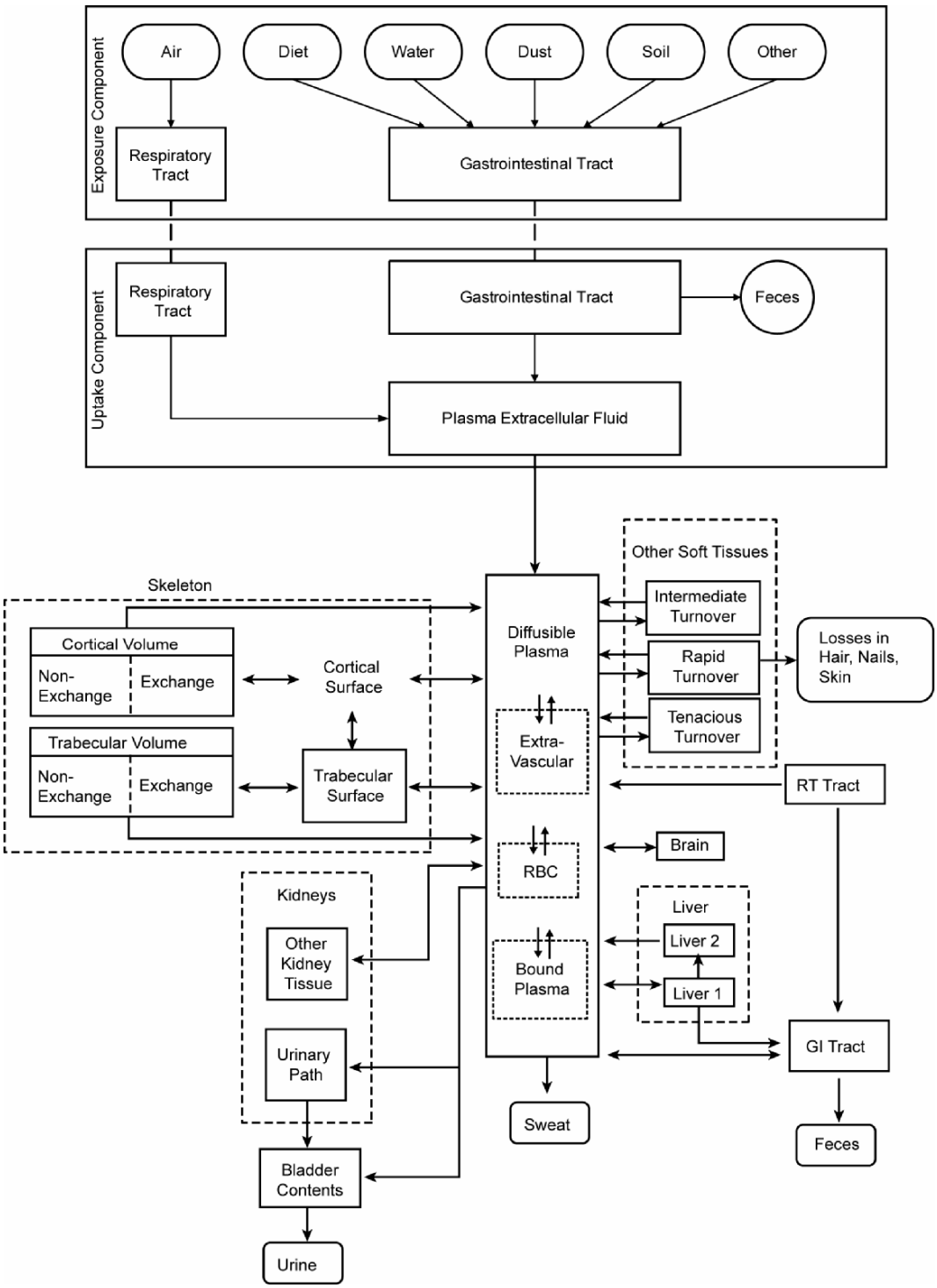

Figure 4-31. Structure of the All Ages Lead Model. The AALM adds a comprehensive exposure component and an uptake component to a revised and recoded version of the Leggett model to produce a model with fully selectable exposure, uptake, and biokinetic parameters.

meat; and corresponding food intakes for each food type (μg food/day). Lead intake from drinking water is calculated from concentrations (μg/L) in tap water (first draw and/or flushed), fountain water, and/or bottled water; and corresponding source water intake rates (L/day).

The dust exposure module accepts input values for dust concentrations (μg/g) in various settings (e.g., residential, school, recreational, occupational) or dust loadings (μg/m^2) and corresponding dust ingestion rates (μg dust/day) or contact rates (m^2/day), the Pb ingestion rate for a given loading being calculated as the product of loading and contact rate. Pica ingestion for soil and/or paint chips can be simulated with input values for Pb levels in soil (μg/g) or paint (μg/cm^2) and corresponding pica ingestion rates (g soil/day, cm^2 paint/day). Dermal exposure to Pb in dust can also be simulated with input values for dust Pb level (μg/g), dust loading on the skin (mg/cm^2), and skin exposure rate (cm^2/day).

Calculated Pb intakes for each exposure pathway are summed to calculate total intakes (μg/day) to the respiratory tract, gastrointestinal tract, and dermal pathway, respectively. The exposure model time step is 1 day (the smallest time interval for a single exposure event).

Biokinetics Module. The biokinetics module of the AALM is based on Leggett (1993) with the following modifications and enhancements:

1. A simulation of dermal absorption is implemented that calculates transfer of Pb from the skin to the central plasma compartment, as a function of rate of dermal contact with Pb (μg/day) and a dermal absorption fraction.

2. Male and female growth algorithms for body weight, soft tissues, and cortical and trabecular bone are implemented, based on O'Flaherty (1993, 1995). This allows simulation of tissue growth and volumes, as well as Pb concentrations in all tissues simulated.

3. A simulation of maternal-fetal transfer is implemented that simulates Pb levels in fetal tissues, and establishes blood and tissue Pb levels for a postnatal simulation. This provides a means for multigeneration simulation of exposure and Pb biokinetics.

4.4.9 Model Comparisons

Table 4-17 summarizes the major features of various models of human exposure that predict tissue Pb burdens. The slope factor models give similar predictions of quasi-steady state

Table 4-17. Summary of Models of Human Exposure that Predict Tissue Distribution of Lead

Model	Age Range	Exposure Pathways	Exposure Time Step	Biokinetics Simulation	Biomarkers Predicted	Variability and Uncertainty Simulation
U.S. Environmental Protection Agency IEUBK Model White et al. (1998)	0-7 yr	Air Diet Soil/dust Water Other	1 year	Multicompartmental	Blood Pb	Variability: blood Pb GSD_i Variability/uncertainty: MCA (Griffin et al., 1999b)
U.S. Environmental Protection Agency AALM (2005)	0-90 yr	Air Diet Soil/dust Water Other	1 day	Multicompartmental	Blood Bone Brain Fetus Kidney Liver Urine	Variability and uncertainty determined by independent assessment of multiple runs of the model.
Leggett (1985)	0-Adult	Intakes (inhaled, ingested, injected)	1 day	Multicompartmental	Blood Bone Brain Kidney Liver Urine	NA
O'Flaherty (1993, 1995)	0-Adult	Air Diet Soil/dust Water Other	1 year (code supports 1 day)	Multicompartmental	Blood Bone Brain Kidney Liver Urine	Beck et al. (2001)

Table 4-17 (cont'd). Summary of Models of Human Exposure that Predict Tissue Distribution of Lead

Model	Age Range	Exposure Pathways	Exposure Time Step	Biokinetics Simulation	Biomarkers Predicted	Variability and Uncertainty Simulation
U.S. Environmental Protection Agency ALM Maddaloni et al. (2005)	Adult	Soil (supports other pathways)	>3 months (quasi-steady state)	Uptake slope factor	Blood	Variability: blood Pb GSD_i
California Environmental Protection Agency, Carlisle and Wade (1992)	Child Adult	Air Diet Soil/dust Water	>3 months (quasi-steady state)	Intake slope factor	Blood	Variability: blood Pb GSD_i
Bowers et al. (1994)	Adult	Air Soil/dust Water	<3 months (quasi-steady state)	Uptake slope factor	Blood	Variability: blood Pb GSD
Stern (1994, 1996)	Child Adult	Dust/soil	>3 months (quasi-steady state)	Intake slope factor	Blood	Variability: blood Pb GSD_i: MCA

blood Pb concentration when similar inputs and parameter values were applied to each model (Maddaloni et al., 2005). Of the models presented in Table 4-17, Bowers et al. (1994) and U.S. EPA (2003c) implement uptake slope factors. The slope factors used in both models (~0.4 μg/dL per μg Pb/day) are similar to biokinetic slope factors predicted from the O'Flaherty model (0.65 μg/dL per μg Pb uptake/day) and Leggett model (0.43 μg/dL per μg Pb uptake/day) for simulations of adult exposures (Maddaloni et al., 2005). A review of reported intake slope factors relating medium-specific exposures and blood Pb concentrations derived from epidemiologic studies can be found in the 1986 AQCD and in Abadin and Wheeler (1997).

Lead uptake-blood Pb concentration relationships in children, predicted by the IEUBK, Leggett, and O'Flaherty models are shown in Figure 4-32. In the range of uptakes shown (0.1 to 100 μg Pb absorbed/day), nonlinearity of the relationship is apparent in both the Leggett and the O'Flaherty models simulations. This reflects assumptions in each model regarding the limited capacity of red blood cells to take up Pb, which has also been observed in humans (Bergdahl et al., 1997a, 1998, 1999; Manton et al., 2001; Smith et al., 2002; see Section 4.3.1 for further discussion of curvilinear relationship between Pb intake and blood Pb concentration). Regression slopes (μg/dL blood per μg/day uptake) for the predictions ≤10 μg/dL are: Leggett model, 0.88; IEUBK model, 0.36; O'Flaherty model, 0.29. The models predict an average blood Pb concentration of 10 μg/dL for the age range 2 to 3 years, in association with average Pb uptakes (μg/day) for the same period of approximately: Leggett model, 12; IEUBK model, 29; O'Flaherty model, 36.

A similar comparison of uptake-blood Pb concentration relationships predicted in adults is shown in Figure 4-33. Regression slopes for adults predicted by the Leggett and O'Flaherty models (at blood Pb concentrations ≤10 μg/dL) are more similar for adults (Leggett model, 0.54; O'Flaherty model, 0.72) than for children (see Figure 4-32 versus Figure 4-33). The models predict an average blood Pb concentration of 10 μg/dL for the age range 31 to 32 years, in association with average Pb uptakes, for the same period, of ~18 and 13 μg/day, Leggett and O'Flaherty models, respectively. The nonlinearity in both children and adults is due largely to assumptions made in the models about the limited capacity of red blood cells to take up Pb at concentrations above 15 to 20 μg/dL. The IEUBK model (for children) does not include this nonlinearity feature.

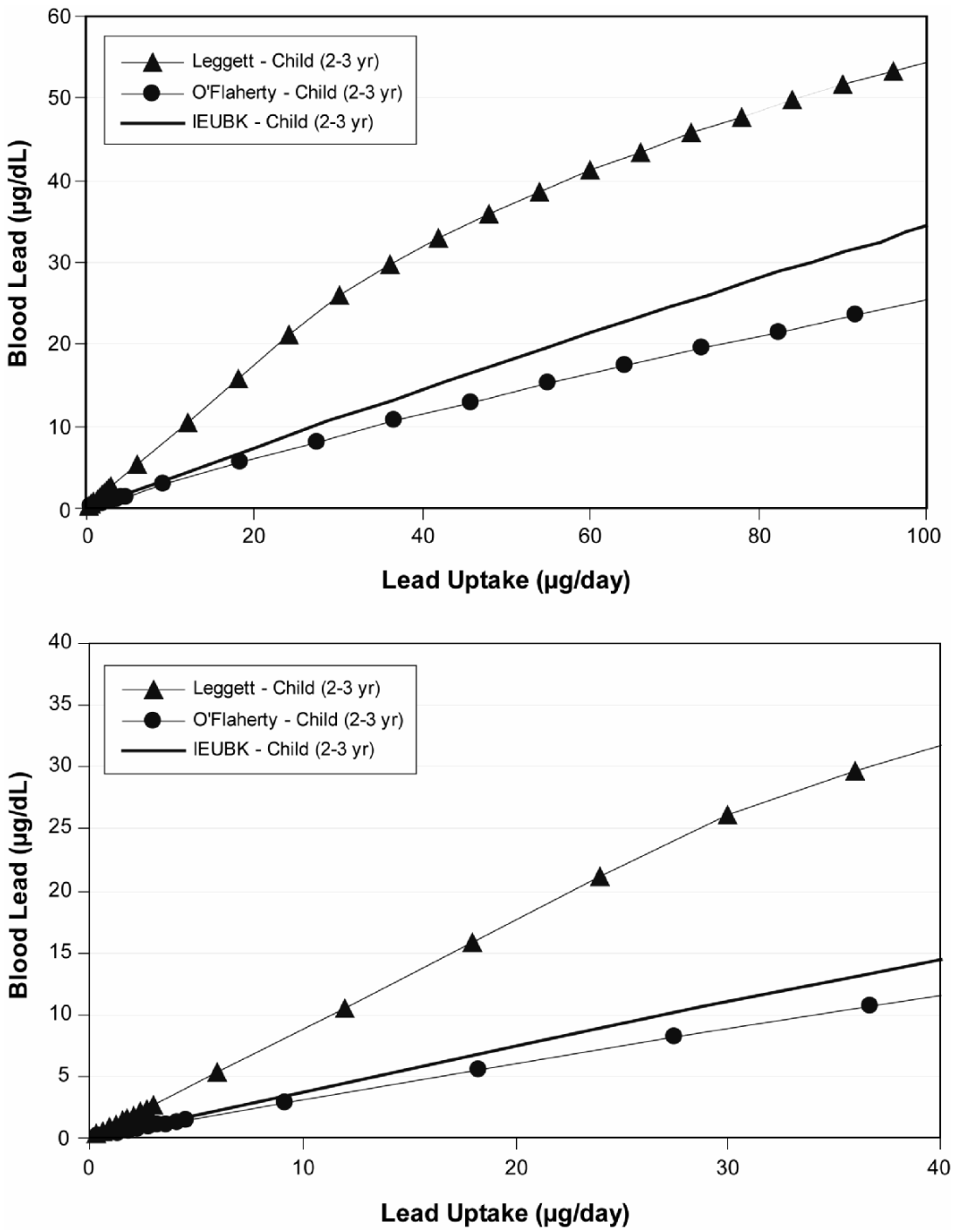

Figure 4-32. Model comparison of predicted lead uptake—blood lead concentration relationship in children. In the range of uptakes shown, the nonlinearity of the relationship is apparent in the Leggett and O'Flaherty Models simulations, reflecting the simulation of the limited capacity of red blood cells to take up lead. Regression slopes (µg/dL blood per µg/day uptake) for the predictions ≤10µg/dL are: Leggett Model, 0.88; IEUBK Model, 0.36; O'Flaherty Model, 0.29.

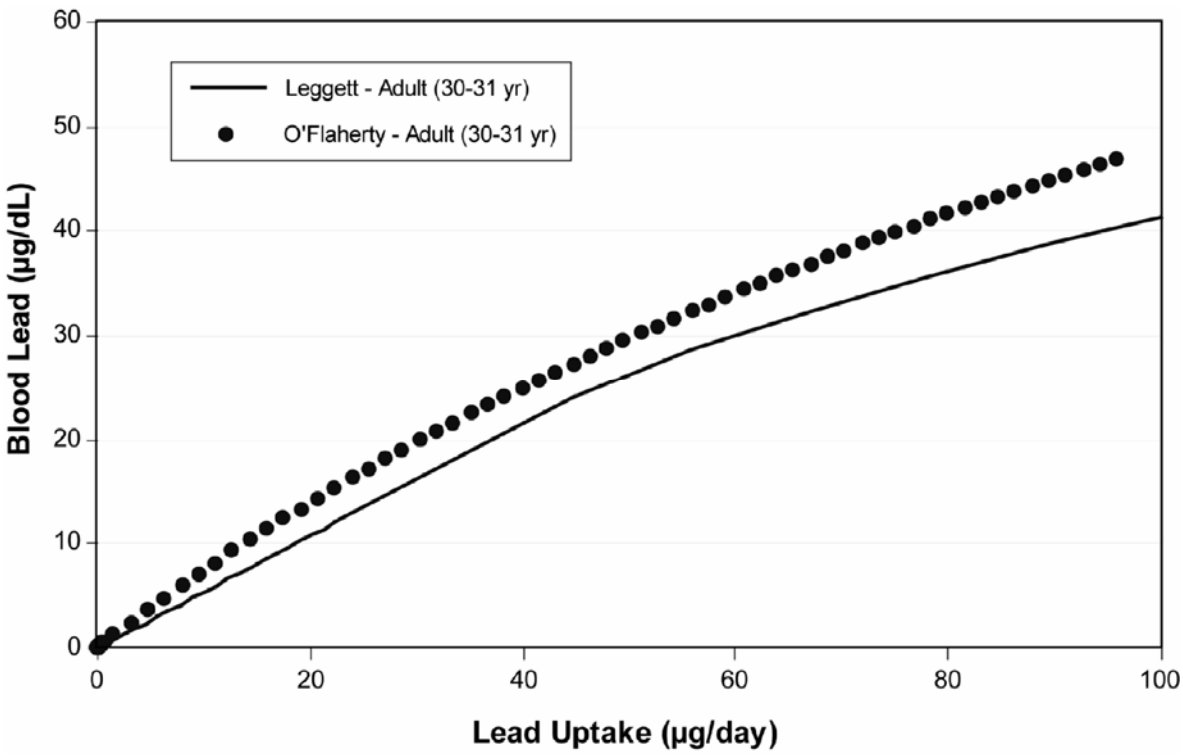

Figure 4-33. Model comparison of predicted lead uptake—blood lead concentration relationships in adults. The nonlinearity of the relationship is apparent in both the Leggett and O'Flaherty Models. Regression slopes (µg/dL blood per µg/day uptake) for the predictions ≤10µg/dL are: Leggett Model, 0.54; O'Flaherty Model, 0.72.

Comparisons of predicted bone and soft tissue Pb burdens are shown in Figure 4-34. Leggett and O'Flaherty models predict bone Pb burdens. Both the Leggett and O'Flaherty models predict a bone Pb burden in adults of ~90 and 98% of total body burden, respectively. Regression slopes (mg Pb in bone per µg uptake/day) are 1.2 for the Leggett model and 2.1 for the O'Flaherty model.

Figures 4-35 and 4-36 compare model predictions for blood Pb concentration for hypothetical childhood or adult Pb exposures. The hypothetical child (Figure 4-35) has a blood Pb concentration of 2 µg/dL at age 2 years and then experiences a 1-year exposure to 100 µg Pb/day. All three models (Leggett, IEUBK, and O'Flaherty) predict a similar temporal pattern of increase in blood Pb concentration at the start of exposure, then attainment of a quasi-steady state, followed by a decrease in blood Pb concentration, with fast and slower phases of the

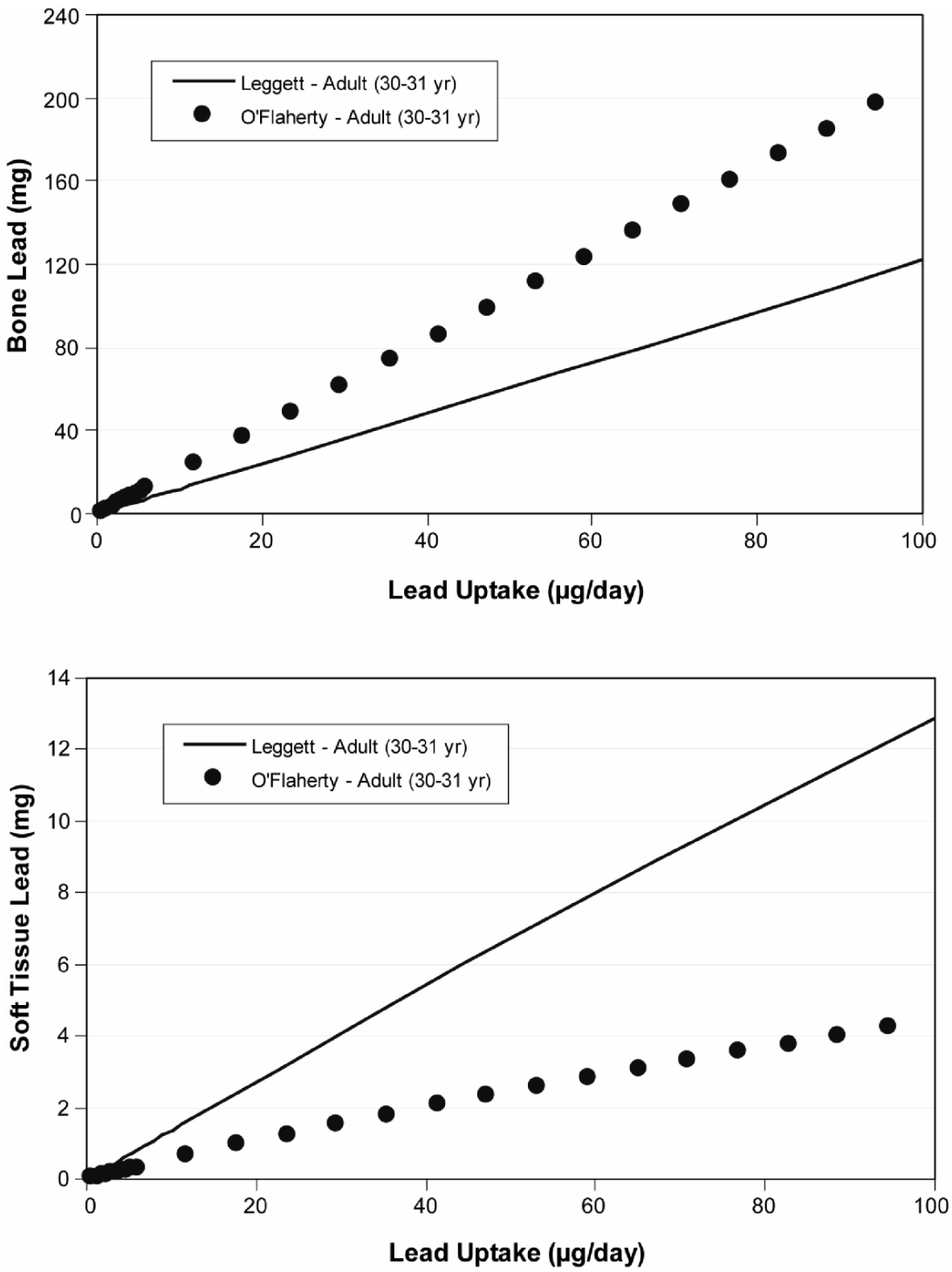

Figure 4-34. Model comparison of predicted of lead uptake—bone and soft tissue lead burden relationship in adults. Both the Leggett and O'Flaherty Models predict a bone lead burden of ~90% and 98% of total body burden, respectively. Soft tissue burdens shown include blood. Regression slopes (mg lead per µg uptake/day) for uptake-bone burden relationship is: Leggett, 1.2; O'Flaherty Model, 2.1.

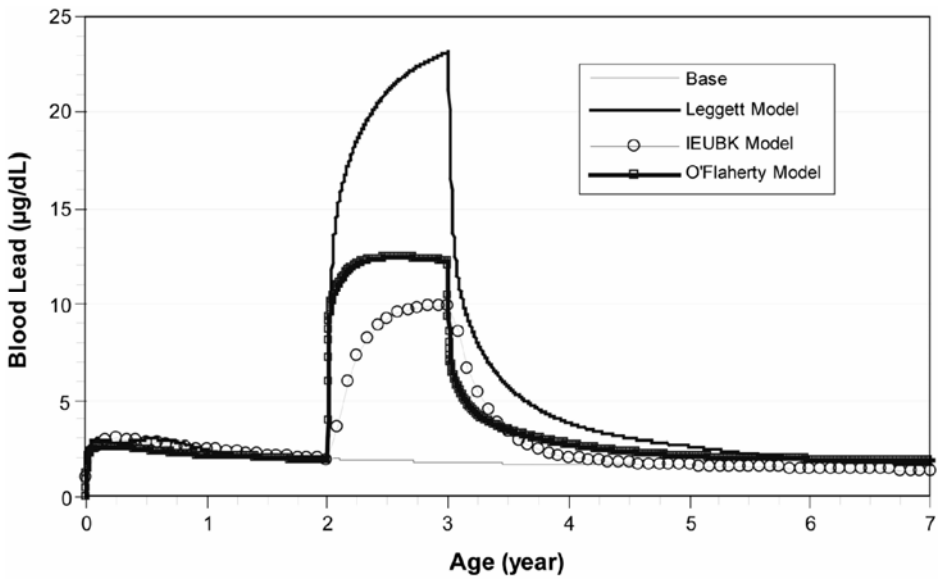

Figure 4-35. Comparison of model predictions for childhood lead exposure. The simulations are of a hypothetical child who has a blood lead concentration of 2 µg/dL at age 2 years, and then experiences a 1-year exposure to 100 µg lead/day. Default bioavailability assumptions were applied in all three models.

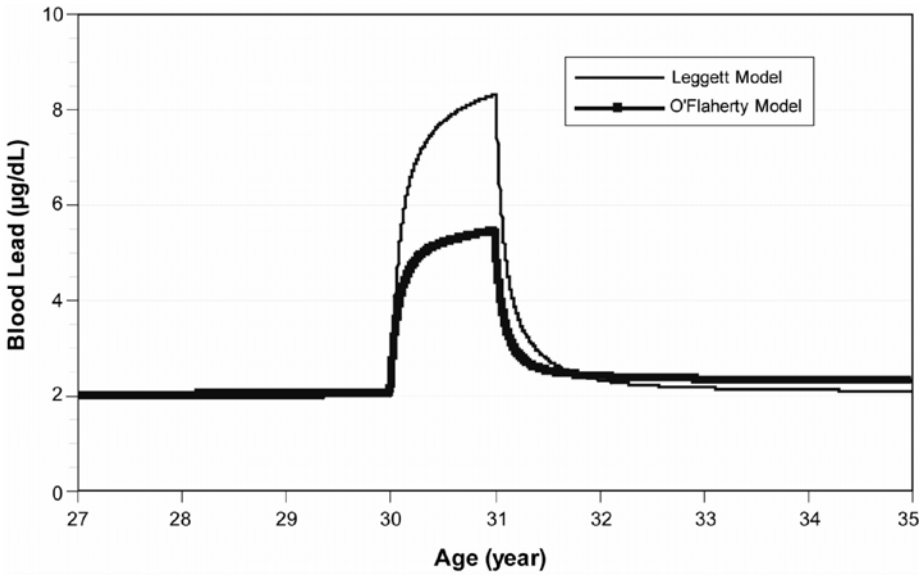

Figure 4-36. Comparison of model predictions for adult lead exposure. The simulations are a hypothetical adult who has a blood lead concentration of 2 µg/dL at age 30 years and then experiences a 1-year exposure to 100 µg lead/day. Default bioavailability assumptions were applied in the Leggett and O'Flaherty models.

decline in blood Pb concentration after the exposure ceases. However, differences in the predicted kinetics of the blood Pb changes and the predicted quasi-steady state blood Pb concentrations are evident. For this hypothetical scenario, the Leggett model predicts the highest blood Pb concentrations (23 µg/dL) compared to the O'Flaherty (12 µg/dL) and IEUBK (10 µg/dL) models. These differences are not solely the result of different values for the absorption fraction in 2 to 3 year old children: Leggett model, 30%; O'Flaherty model, 45% (descending from 49% at age 2 years to 39% at age 3 years); IEUBK model, 25% (at a soil Pb intake of 100 µg/day). A similar pattern is evident in the simulation of the same exposure (100 µg/day for 1 year) in an adult (age 30 years; Figure 4-36). The Leggett model predicts a quasi-steady state blood Pb concentration of ~8.2 µg/dL and the O'Flaherty model predicts 5.4 µg/dL. However, most of this difference can be attributed to the different absorption fraction values used for adults in the two models; 15% in the Leggett model and 8% in the O'Flaherty. A comparison of predictions of quasi-steady state blood Pb concentrations from various models was reported in Maddaloni et al. (2005). Results of comparisons between the U.S. ALM, Leggett model and O'Flaherty model are presented in Table 4-18. When similar exposure inputs are used in the three models, similar blood Pb concentrations are predicted. Much of the difference between the Leggett model and ALM predictions can be ascribed to differences in assumed bioavailability of Pb in soil: 12% in the U.S. EPA ALM, and 15% in the Leggett model.

4.4.10 Conclusions and Future Directions

Modeling of relationships between Pb exposures and Pb levels in tissues has advanced considerably during the past 25 years or so. Three mechanistic models have been developed and evaluated to varying degrees for predicting associations between exposure and body burden (IEUBK model, Leggett model, O'Flaherty model). A fourth model, the All Ages Lead Model, is still under development and may resolve some of the issues regarding discrepancies between other models, while at the same time adding new features directly applicable to risk assessment.

The IEUBK model has had the most extensive application in the regulatory context, as EPA guidance recommends that, where possible, risk estimates for residential exposures to Pb at hazardous waste sites be based on IEUBK model predictions of blood Pb concentrations

Table 4-18. Inputs and Results of Simulations Comparing the U.S. EPA Adult Lead Methodology (ALM) With Multicompartmental Models

Parameters	ALM	Rabinowitz model	Bert model	Leggett model	O'Flaherty model
Soil Pb concentration	1000 µg/g	1000 µg/g [a,b]	1000 µg/g [a,c]	1000 µg/g [a,c]	1000 µg/g [a,c]
IR_S	0.05 g/day	0.05 g/day [a]	0.05 g/day [a]	0.05 g/day [a]	0.05 g/day [a]
AF	0.12	0.12[a]	0.12 (0.08)[d]	0.12 (0.15)[d]	0.08
Baseline blood Pb	2 µg/dL	2 µg/dL[e]	2 µg/dL[f]	2 µg/dL[g]	2 µg/dL[h]
Exposure frequency	5 days/week (260 days/year)[i]	5 days/week (260 days/year)	5 days/week (260 days/year)	5 days/week (260 days/year)	5 days/week (260 days/year)
Exposure duration	NA	365 days	365 days	17–45 years	17–45 years[j]
Predicted quasi-steady state PbB (µg/dL)					
Soil Pb: 1000 µg/g	3.7	4.1	4.0 (2.6)[k]	4.1 (4.9)[k]	4.6
Soil Pb: 10,000 µg/g	19	23	21 (14)[k]	23 (29)[k]	24

[a]Not a parameter in the model.
[b]Simulated as an increment in daily uptake of 6 µg/day (i.e., 1000 × 0.05 × 0.12) above baseline.
[c]Simulated as an increment in daily intake of 50 µg/day (i.e., 1000 × 0.05) above baseline.
[d]Default values are shown in parenthesis.
[e]A daily uptake of 4.1 µg/day yielded a quasi-steady state PbB of 2 µg/dL.
[f]A daily intake of 38.7 µg/day yielded a quasi-steady state PbB of 2 µg/dL.
[g]A daily intake that varied from 12 to 25 µg/day yielded pre-adult PbBs within the ranges reported from Phase I of NHANES III (Brody et al. 1994) and an adult PbB of 2 µg/dL.
[h]Setting all Pb concentrations and intakes to null and food Pb ingestion by adults born in 1980 to 25 µg/day yielded pre-adult PbBs within the ranges reported from Phase I of NHANES III (Brody et al. 1994) and an adult PbB of 2 µg/dL.
[i]The default exposure frequency for the ALM is 219 days/year; however, the assumption of 260 days/year in the simulations would not change the outcome of the model comparisons.
[j]Adults born in 1980.
[k]Predictions based on the default value for the AF are shown in parenthesis.

in children. Although, these models are constructed very differently (e.g., the O'Flaherty biokinetics model has only 17 Pb parameters, compared to 65 in the Leggett biokinetics model, and 47 in the IEUBK biokinetics model), the three models yield remarkably similar predictions of blood Pb concentration for similar hypothetical exposure scenarios. The three models predict similar kinetics of change in blood Pb concentrations in association with a change in Pb exposure (e.g., Figures 4-35 and 4-36). Both the Leggett and O'Flaherty models predict similar rates of Pb accumulation in bone, for the same rates of uptake of Pb into the body. Predictions of quasi-steady state blood Pb concentrations for the scenarios are simulated in Figures 4-35 and 4-36 and differ across models by a factor of ~2. This magnitude of difference is substantial in the context of certain regulatory uses of the models (e.g., for establishing cleanup goals at hazardous waste sites); however, it is not surprising, given the various approaches taken to reduce the complex biokinetics of Pb to tractable, and relatively simple, mathematical expressions.

Several major challenges remain to be confronted in further developing our ability to simulate Pb exposure-tissue level relationships in real individuals or populations. The three earlier mechanistic models described above do not simulate the kinetics of Pb in pregnancy or in senescence (e.g., menopause). Only one of these three earlier models (Leggett) simulates Pb levels in brain, a potential target organ for Pb toxicity. None of the models have been rigorously evaluated for accuracy of predictions of bone Pb levels in humans, for which there is a rapidly expanding set of observations of importance to dose-response assessment. Of great importance for regulatory uses of the models, for example, is the need for more rigorous quantitative assessment of confidence (i.e., uncertainty) in model predictions. To date, such assessments have not been applied uniformly in a manner that allows cross-model comparisons of confidence for specific regulatory uses.

The IEUBK Model has undergone the most extensive and thoroughly reported evaluation of a regulatory use of the model, i.e., (a) quantitative evaluation of predicted distributions of blood Pb concentrations in children who live in areas for which cross-sectional measurements of environmental Pb levels were available and (b) independent verification of the IEUBK model implementation code (Hogan et al., 1998; Zaragoza and Hogan, 1998). However, a similar level of evaluation of the Leggett and O'Flaherty models has not been reported, although specific predictions of the models have been evaluated against observations (e.g., experimentally-observed kinetics of change in blood Pb following a change in intakes).

To a large extent, the important information gap regarding evaluation of model confidence derives from a lack of observational data and/or public access to observational data on which predictions could be evaluated. An additional challenge for applications of the models in a regulatory context relates to uncertainties in exposure data from which exposure model inputs are derived. Model development and uncertainty assessment could be substantively advanced by assembling verified (for accuracy) sets of data on Pb biokinetics against which models could be uniformly evaluated. Examples of the types of data that would be valuable include data on the kinetics of change in blood or tissue Pb concentrations, or stable Pb isotope ratios, in response to a change in exposure. Also, access to large data bases that include reported Pb exposure measurements for various media that are paired with blood or tissue Pb measurements for individuals affected by pertinent exposure scenarios would also be extremely valuable for cross-model evaluations.

4.5 SUMMARY

At the time of the 1986 Lead AQCD, it was recognized that Pb distributed to and accumulated in several bone compartments which exhibited differing mobility profiles. It was also recognized that a larger fraction of total body burden of Pb is found in the bones of adults relative to children. The possibility of bone Pb serving as a source of long-term internal exposure was considered. Several models of Pb pharmacokinetics of Pb in humans were developed to simulate the multiphasic elimination kinetics of Pb from blood, bone, and soft tissues (Marcus, 1985a,b,c; Rabinowitz et al., 1976). New studies have been published on the kinetics of Pb movement into and out of bone which demonstrate the importance of bone Pb stores as a source of Pb to the blood in retired Pb workers and during pregnancy. Additional information regarding Pb absorption, distribution, and elimination in humans is available.

The main pathway of intake of Pb in the general human population is ingestion (diet, ingestion of surface dust). Inhalation can be an important pathway in some occupational settings. Dermal absorption of inorganic Pb compounds is generally considered to be much less than absorption by inhalation or oral routes of exposure; however, few studies have provided quantitative estimates of dermal absorption of inorganic Pb in humans, and the quantitative significance of the dermal absorption pathway as a contributor to Pb body burden in humans

remains an uncertainty. Absorption of ingested Pb is affected by numerous factors, including the individual's age, diet, and nutritional status, as well as chemical and physical properties of Pb. Lead absorption appears to be increased by both iron and calcium deficiency. Fasting also increases the absorption of ingested Pb (Blake et al., 1983; Heard and Chamberlain, 1983; James et al., 1985; Rabinowitz et al., 1980; Maddaloni et al., 1998). Gastrointestinal (GI) absorption of Pb in humans may be a capacity limited process such that the fraction of ingested Pb that is absorbed may decrease with increasing rate of Pb intake. The available studies to date, however, do not provide a firm basis for discerning if the GI absorption of Pb in humans is limited by dose. The size of ingested Pb particles also affects GI absorption, with absorption decreasing with increasing particle size (Ruby et al., 1999; Healy et al., 1992). Exposures to airborne inorganic Pb are usually in the form of particulate aerosols. Deposition and clearance of Pb particles from the respiratory tract are affected by numerous factors, including the individual's age and activity, particle size, and physical-chemical properties of the inhaled Pb.

In general, the burden of Pb in the body may be viewed as divided between a dominant slow compartment (bone) and a smaller fast compartment (soft tissues). In human adults, more than 90% of the total body burden of Pb is found in the bones, whereas bone Pb accounts for ~70% of the body burden in children (Barry, 1975). The highest soft tissue concentrations in adults also occur in liver and kidney cortex (Barry, 1975; Gerhardsson et al., 1986, 1995b; Gross et al., 1975; Oldereid et al., 1993). Lead in blood (i.e., plasma) exchanges with both of these compartments. The contribution of bone Pb to blood changes with the duration and intensity of the exposure, age, and various physiological variables (e.g., nutritional status, pregnancy, and menopause).

Lead accumulates in bone regions having the most active calcification at the time of exposure. Lead accumulation is thought to occur predominantly in trabecular bone during childhood and in both cortical and trabecular bone in adulthood (Aufderheide and Wittmers, 1992). Lead concentrations in bone increase with age throughout the lifetime, indicative of a relatively slow turnover of Pb in adult bone (Barry 1975, 1981; Gross et al., 1975; Schroeder and Tipton, 1968). Some bones (i.e., mid femur and pelvic bone) increase in Pb content plateaus at middle age and then decreases at higher ages (Drasch et al., 1987). This decrease is most pronounced in females and may be due to osteoporosis and release of Pb from resorbed bone to blood (Gulson et al., 2002). Lead in adult bone can serve to maintain blood Pb levels long after

external exposure has ceased (Fleming et al., 1997; Inskip et al., 1996; Kehoe, 1987; O'Flaherty et al., 1982; Smith et al., 1996). During pregnancy, bone Pb can also serve as a Pb source as maternal bone is resorbed for the production of the fetal skeleton (Franklin et al., 1997; Gulson et al., 1997, 1999b, 2003).

The highest soft tissue concentrations in adults also occur in liver and kidney cortex (Barry, 1975; Gerhardsson et al., 1986, 1995b; Gross et al., 1975; Oldereid et al., 1993). In contrast to Pb in bone, which accumulates with continued exposure in adulthood, Pb concentrations in soft tissues (e.g., liver and kidney) are relatively constant in adults (Barry 1975; Treble and Thompson 1997), reflecting a faster turnover of Pb in soft tissue relative to bone. Pb in soft tissues exists predominantly bound to protein. High affinity cytosolic Pb binding proteins (PbBPs) have been identified in rat kidney and brain (DuVal and Fowler 1989; Fowler 1989). Other high-affinity Pb binding proteins (Kd ~14 nM) have been isolated in human kidney, two of which have been identified as a 5 kD peptide, thymosin 4 and a 9 kD peptide, acyl-CoA binding protein (Smith et al., 1998a).

Lead in blood is found primarily (~99%) in the red blood cells (Bergdahl et al., 1997b, 1998, 1999; Hernandez-Avila et al., 1998; Manton et al., 2001; Schutz et al., 1996; Smith et al., 2002). δ-aminolevulinic acid dehydratase (ALAD) is the primary binding ligand for Pb in erythrocytes (Bergdahl et al., 1997b, 1998; Sakai et al., 1982; Xie et al., 1998). Lead binding to ALAD is saturable; the binding capacity has been estimated to be ~850 μg/dL red blood cells (or ~40 μg/dL whole blood) and the apparent dissociation constant has been estimated to be ~1.5 μg/L (Bergdahl et al., 1998). It has been suggested that the small fraction of Pb in plasma (<0.3%) may be the more biologically labile and toxicologically active fraction of the circulating Pb. Several authors have proposed that Pb released from the skeleton was preferentially partitioned into serum compared with red cells (Cake et al., 1996; Hernandez-Avila et al., 1998; Tsaih et al., 1999). Approximately 40 to 75% of Pb in the plasma is bound to proteins, of which albumin appears to be the dominant ligand (Al-Modhefer et al., 1991; Ong and Lee, 1980). Lead in serum that is not bound to protein exists largely as complexes with low molecular weight sulfhydryl compounds (e.g., cysteine, homocysteine) and other ligands (Al-Modhefer et al., 1991).

Blood Pb concentration is extensively used in epidemiologic studies as an index of exposure and body burden due mainly the feasibility of incorporating its measurement into

human studies relative to other potential dose indicators, e.g., Pb in kidney, plasma, urine, or bone. A single blood Pb measurement may not distinguish between a history of long-term lower level Pb exposure from a history that includes higher acute exposures (Mushak, 1998).
An additional complication is that the relationship between Pb intake and blood Pb concentration is curvilinear; that is, the increment in blood Pb concentration per unit of Pb intake decreases with increasing blood Pb concentration, both in children (Lacey et al., 1985; Ryu et al., 1983; Sherlock and Quinn, 1986) and in adults (Kehoe, 1987; Laxen et al., 1987; Pocock et al., 1983; Sherlock et al., 1982, 1984). In general, higher blood Pb concentrations can be interpreted as indicating higher exposures (or Pb uptakes); however, they do not necessarily predict higher body burdens. Similar blood Pb concentrations in two individuals (or populations) do not necessarily translate to similar body burdens or similar exposure histories. The disparity in the kinetics of blood Pb and body burden may have important implications for the interpretation of blood Pb concentration measurements in some epidemiology studies, depending on the health outcome being evaluated.

In addition to blood Pb, hair and urine Pb have also been used as biomarkers of Pb exposure. An empirical basis for interpreting hair Pb measurements in terms of body burden or exposure has not been firmly established. Hair Pb measurements are subject to error from contamination of the surface with environmental Pb and contaminants in artificial hair treatments (i.e., dyeing, bleaching, permanents) and, as such, are relatively poor predictor of blood Pb concentration, particularly at low levels (<10 to 12 μg/dL). Urine Pb concentration measurements provide little reliable information, unless they can be adjusted to account for unmeasured variability in urine flow rate (Araki et al., 1990). Similar to blood Pb concentration measurements, urinary Pb excretion measured in an individual at a single point in time will reflect the recent exposure history. As a result, measurement of urinary Pb may serve as a feasible surrogate for plasma Pb concentration, and may be useful for exploring dose-response relationships for effect outcomes that may be more strongly associated with plasma Pb concentration than Pb body burden.

Several new studies have investigated the relationship between Pb exposure and blood Pb in children. These studies support the concept that contact with Pb in surface dust (interior and exterior) are a major contributor to Pb intake in children. In the meta-analysis by Succop et al. (1998), the most common exposure pathway influencing blood Pb concentration was exterior

soil, operating through its effect on interior dust Pb and hand Pb. Using a structural equation model, Lanphear and Roghmann (1997) also found the exposure pathway most influential on blood Pb was interior dust Pb loading, directly or through its influence on hand Pb. Both soil and paint Pb influenced interior dust Pb; with the influence of paint Pb greater than that of soil Pb. Interior dust Pb loading also showed the strongest influence on blood Pb in a pooled multivariate regression analysis (Lanphear et al., 1998).

New information on Pb biokinetics, bone mineral metabolism, and Pb exposures has led to refinements and expansions of earlier mechanistic models of Pb biokinetics. In particular, three pharmacokinetic models are currently being used or considered for broad application in Pb risk assessment: (1) the Integrated Exposure Uptake BioKinetic (IEUBK) model for Pb in children developed by EPA (U.S. Environmental Protection Agency, 1994a,b; White et al., 1998); (2) the Leggett model, which simulates Pb kinetics from birth through adulthood (Leggett, 1993); and (3) the O'Flaherty model, which simulates Pb kinetics from birth through adulthood (O'Flaherty, 1993, 1995). The above three models have been individually evaluated, to varying degrees, against empirical physiological data on animals and humans and data on blood Pb concentrations in individuals and/or populations (U.S. Environmental Protection Agency, 1994a,b; Leggett, 1993; O'Flaherty, 1993). In evaluating models for use in risk assessment, exposure data collected at hazardous waste sites, where exposures to contaminated soils are the dominant contributors to exposure, have been used to drive model simulations of corresponding blood Pb distributions (Bowers and Mattuck, 2001; Hogan et al., 1998; TerraGraphics, Inc, 2000, 2001). The exposure module in the IEUBK model makes this type of evaluation feasible. These empirical comparisons have shown that agreement or disparity between IEUBK model predictions and observed blood Pb concentrations at specific locations is influenced by numerous factors, including (a) the extent to which the exposure and blood Pb measurements are adequately matched and (b) site-specific factors (e.g., behavior patterns, soil characteristics, bioavailability) that may affect Pb intake or uptake in children. Exposure-biokinetics models serve not only to illustrate exposure-blood-body burden relationships, but also provide a means for making predictions about them that can be tested experimentally or in epidemiologic studies.

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5. TOXICOLOGICAL EFFECTS OF LEAD IN LABORATORY ANIMALS AND IN VITRO TEST SYSTEMS

5.1 INTRODUCTION

As noted in Chapter 1, U.S. EPA air quality criteria documents evaluate scientific knowledge of relationships between pollutant concentrations and their effects on the environment and public health. Chapters 2 and 3 of this document discussed the chemistry and physical properties of lead (Pb); sources, emissions, transport, and deposition of Pb; and environmental concentrations and pathways to human exposure. Chapter 4 discussed biokinetics of external Pb exposure impacts on internal distribution of lead to body tissues and models of human exposure that predict tissue distribution of Pb. This chapter (Chapter 5) assesses information regarding the toxicological effects of Pb in laboratory animals and in vitro test systems. Emphasis is not only placed here on qualitative characterization of various Pb-induced effects, but also on attempts to define dose-effect relationships for key health effects thought likely to occur at ambient exposure levels encountered by the general population of the United States. Chapter 6 follows with a discussion of epidemiologic studies of ambient Pb-exposure effects. The environmental effects of Pb are then discussed in Chapter 7. Lastly, Chapter 8 provides an overall integrative synthesis of information on Pb exposures, health effects and their potential public health significance, and environmental (especially ecologic) effects of Pb.

The framework used here for assessing the toxicologic effects of Pb is subdivided mainly according to organ systems. As noted in the last previous Lead Air Quality Criteria Document (Lead AQCD) published in 1986, this facilitates presentation of the information, but it must also be stressed that all systems are interdependent, functioning in delicate concert to preserve the physiological integrity of the whole organism.

The information discussed in this chapter is derived from a very wide body of literature on studies in laboratory animals and in vitro test systems of animal cell lines and organ systems that may mimic responses in intact animals. This chapter is not intended to be a compendium of all that is known about Pb effects; rather, it is an update assessment of the reported biological effects from the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a), the Addendum to that document (Lead Effects on Cardiovascular Function, Early Development, and

Stature) (U.S. Environmental Protection Agency, 1986b), and the Supplement to the 1986 Addendum (U.S. Environmental Protection Agency, 1990). The historical Pb literature is briefly summarized at the opening of each section or subsection and is intended as a very concise overview of previous work. The reader should refer to the previous documents listed above for more detailed discussion of the literature prior to the late 1980s. Each section then continues with brief, evaluative discussion of key studies published since 1986. Longer discussions of newly available studies are included where warranted. Sections also include comparisons of findings from the 1986 AQCD to those derived from the new data and statements of key bottomline conclusions regarding Pb effects on given types of health endpoints. More detailed descriptive summaries of newly available studies and results are provided in Annex AX5.

5.2 EFFECTS OF LEAD ON HEME SYNTHESIS

5.2.1 Effects of Lead on Erythrocyte Biology and Function

Lead poisoning is one of the most common acquired environmental diseases, because of physical properties of the metal and its widespread environmental distribution. It is a complex disorder affecting several organs in the body, including developing erythrocytes (red blood cells [RBCs]). Anemia is frequently observed with Pb poisoning and is thought to result from the shortening of erythrocyte life span and the effects of Pb on hemoglobin synthesis. However, the exact mechanisms by which Pb affects the red blood cell (RBC) life span and heme synthesis are not clear. It is postulated that the mechanisms may be due to the effects of Pb on iron uptake and several other interactions of Pb and iron-mediated cellular processes. It has been demonstrated for well over three decades now that conditions of iron deficiency and Pb poisoning can independently occur and coexist. Both conditions are capable of independently producing microcytosis and anemia. In erythrocytes, they affect several cellular processes, and their combined effect on hemotology is an interesting area of study. The increase in Pb absorption in iron deficient rats was first demonstrated in 1972 with several more studies following later, including one by Wright et al. (1998). A common iron-Pb transporter, DMTI, has been postulated that could lead to potential competition for binding sites that could affect intestinal absorption; and DTMI has a higher afficity for Pb. It has also been observed that anemia accompanying the combination of both conditions is more severe and more hypochromic than

uncomplicated iron deficiency anemia. When heme synthesis is inhibited at the final step in heme synthesis, the net effect is that zinc (instead of iron) is incorporated into protoporphyrin, resulting in elevated levels of zinc protoporphyin (ZPP). Both iron deficiency and Pb poisoning are capable of inhibiting heme synthesis at this final step, and combined conditions lead to dramatic ZPP elevations. Lead poisoning also causes increased urinary excretion of porphyrins and 5-aminolevulinic acid (ALA), the first precursor for heme synthesis. Additional evidence for striking similarities between Pb poisoning and acute intermittent porphyria (the disease associated with lesions in the heme biosynthetic enzyme, porphobilinogen deaminase) strongly suggests that one major site of Pb intoxication is the heme biosynthetic pathway.

The 1986 Lead AQCD assessed literature available at that time from both animal and human studies indicating potential effects of Pb intoxication on enzymes and precursors involved in heme synthesis, erythrocyte morphology and function, as well as the influence of these perturbations on the nervous system and vitamin D metabolism and associated physiological process. In summary, those studies reported an association between increased Pb exposure and increased ALAS activity (which is increased in kidney with acute exposure and in spleen with chronic exposure, while it decreased in liver tissue in both the exposure scenarios). The activity of ALAD appeared to be inversely correlated to blood Pb values and was found to be inhibited in several tissues. It was also inferred from several animal studies that the effect of Pb on heme formation involved both ferrochelatase inhibition and impaired mitochondrial transport of iron. Human studies indicated that occupational exposure to Pb results in decreased erythrocyte cell survival and alterations in erythrocyte membrane integrity and energetics. The vast scientific literature on the effects of Pb on various aspects of heme metabolism in diverse organ systems, both in humans and animals, has expanded over the past two decades. This chapter is primarily concerned with discussions of data from animal and in vitro studies, while the human studies are dealt with in Chapter 6.

5.2.2 Effects of Lead on Erythrocyte Functions

The cellular membrane is one of the main targets for toxic effects of heavy metals such as Pb. Anemia, one of the clinical symptoms of severe Pb intoxication, can develop because of impairment of hemoglobin synthesis and damage of erythrocyte membranes by Pb ions. Although the erythrocyte membrane is not as specialized as other cell membranes are, it carries

out important functions common to other cell membranes, such as active and passive transport and the production of ionic and electric gradients. Changes in erythrocyte membrane lipid and protein profiles can alter the membrane fluidity, potentially affecting enzymatic activity and the functionality of receptors and ion channels present on the plasma membrane and can also influence the ionic and molecular composition of intracellular spaces.

Lead Uptake, Binding, and Transport

Studies by Simons (1986a) indicated that the uptake of Pb into human RBCs is a passive process, i.e., it does not require the use of energy in the form of ATP. In addition, Pb may be able to cross the membrane passively in either direction. This process involves anion transport mechanisms, as characteristic anion exchange inhibitors have been found to inhibit passive uptake of Pb by RBCs (Simons, 1986a,b). It has also been shown that the transport of Pb across the membrane depends on the presence of another anion, the bicarbonate ion, and Pb is transported as Pb-carbonate (Simons, 1986a). When Pb enters the cell, it binds mainly to hemoglobin, and the ratio of bound to free Pb in cytoplasm has been estimated to be 6000:1. Simons (1986a,b) carried out studies using citrate buffers, which may cause hemolysis of RBCs. To avoid the influence of a citrate buffer, Sugawara et al. (1990) measured the uptake of Pb into human RBCs by adding Pb directly into plasma. These investigators also found that the transport of Pb across the erythrocyte membrane is energy-independent (passive) and carrier mediated. Little release of Pb from the cells was seen, suggesting absence of any hemolysis of the cells in this protocol. Furthermore, the progressive accumulation of Pb was not observed. More than 98% of the Pb was found accumulated in the cytoplasm in protein-bound form, whereas only 2% was found in the membrane fraction. Sugawara et al. (1990) also reported finding 45 Pb-binding sites on human hemoglobin. On the other hand, studies reported by Bergdahl et al. (1997), using liquid chromatography coupled with inductive plasma mass spectrometry analysis, suggested aminolevulinic acid dehydratase (ALAD), the enzyme involved in the heme synthesis pathway, to be the principle Pb-binding protein, not hemoglobin, as earlier thought.

Additional studies carried out by Simons (1993a) evaluated the transport of Pb into RBCs for cell Pb contents in the range of 1 to 10 μM and showed that ^{203}Pb uptake was mediated by an anion exchanger and that the efflux was mediated through a vanadate-sensitive pathway

identified with the calcium pump (Simons, 1988). He further concluded that the high ratio of RBC to plasma Pb observed in vivo was due to a labile Pb-binding component within the cytoplasm. Simons (1993a) also observed that exit of Pb ions from the RBC was much lower than expected based on his earlier work with erythrocyte ghosts. Utilizing a group of drugs that modify anion exchange and thiol groups in the cytoplasm, Lal et al. (1996) showed that anion exchange mechanisms and thiol groups were critical factors in how Pb stimulates calcium-dependent processes in erythrocytes. Once the role of anion exchanger proteins had been implicated in Pb transport in erythrocytes, Bannon et al. (2000) investigated whether similar anion exchange processes are involved in the uptake and transport of Pb in other cells, such as Madin-Darby canine kidney epithelial cells. Based on a comparative in vitro study using human erythrocytes and canine kidney epithelial cells, these authors reported transport of Pb in kidney epithelial cells, suggesting similar anion exchange involvement.

Erythrocyte Survival, Mobility, and Membrane Integrity

It is well recognized that Pb intoxication interferes with RBC survival by shortening the life span and altering the mobility of erythrocytes; however, the molecular mechanisms behind these effects of Pb on erythrocyte functions are not well understood. The shape and deformability of the human erythrocyte, or RBC, is maintained by several factors, including low concentration of free intracellular Ca^{2+} (<0.1 μM) and a replenished ATP level. An elevated interfacial Ca^{2+} concentration inside the RBC activates the passive ion efflux via a K^+ selective (voltage independent) channel and a concomitant water transport (Gordos effect). Low concentrations of Pb ions can mimic Ca^{2+} and activate the same channel in the RBC.

Intraperitonially injected Pb significantly decreases rat erythrocyte membrane mobility (Terayama et al., 1986), an effect evident to some extent even below blood Pb concentrations of 100 μg/100 mL. This decrease in rat erythrocyte mobility was found simultaneous or prior to changes in hematological parameters such as hemoglobin (Hb) levels and hematocrits (Hct). The same group (Terayama and Muratsugu, 1988) also reported a significant decrease in erythrocyte membrane sialic acid content at the same blood Pb levels with exposure to Pb (20 mM Pb acetate once a week for 5 weeks). Additional studies by the same group reported that other hematological parameters, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC),

were also significantly decreased upon Pb exposure, along with decreased mobility, sialic acid content, and deformability of rat RBCs. However, the blood Pb levels reported in these studies range from 100 to 800 μg/dL and, at best, point out newer mechanistic details of erythrocyte membrane alterations affecting their survival and mobility. Still, it should be noted that these changes were seen to a minor extent even at blood Pb levels <100 μg/dL. It was speculated that Pb-induced decreases in sialic acid content and deformability of RBCs shorten RBC survival time and may lead to anemia in Pb poisoning. Jehan and Motlag (1995) reported that Pb exposure caused significant change in RBC membrane cholesterol and phospholipid contents along with sialic acid. Coexposure to Zn was found to reduce these alterations.

Lead-induced morphological changes in human RBCs were studied by Eriksson and Bering (1993), using electron paramagnetic resonance imaging. These authors reported that Pb ions (a) induced time-dependent changes in MCV and cell shrinkage and (b) inhibited the Gardos effect. Trialkyl-Pb compounds have also been reported to induce hemolytic activity in erythrocytes, with intensity increasing with hydrophobicity of the compounds (Kleszcyńska et al., 1997). Serrani et al. (1997) reported that Pb ions confer protection against RBC lysis in hypotonic low ionic strength media, presumably due to interaction of Pb with certain constituents in the cell membrane. This resistance to erythrocyte lysis was found to significantly increase with Pb (20 to 25 μM) compared to other metals such as Al, Cd, and Zn (Corchs et al., 2001). The Pb-induced reduction in MCV (RBCs derived from umbilical cord) was found to be reversed when the cells were treated with quinidine, an inhibitor of a potassium channel activator, without any effect on resistance to cell lysis, suggesting changes in cell membrane structure. This effect may also be involved in membrane deformability (Mojzis and Nistiar, 2001).

Heavy metals, including Cd, Zn, and Pb, have been found to alter RBC membrane microviscosity and fluidity (Amoruso et al., 1987). These authors labeled RBC membranes with fluorescent lipid probe all trans 1, 6-diphenyl-1,3,5-hexatriene (DPH) and demonstrated increased polarization with increased membrane lipid viscosity upon exposure to heavy metals. They also postulated that such alterations in cell membrane lipid, and possibly also protein fluidity, may contribute to abnormal cellular function. Similar changes in RBC fluidity were observed in the RBC collected from workers exposed to Pb (Cook et al., 1987). The RBC ghost membranes isolated from Pb-exposed workers exhibited a significant increase in the phosphotidylcholine to phosphotidylethanolamine ratio (an established correlate of membrane

fluidity) along with an increase in RBC cholesterol levels, as also reported by Jehan and Motlag (1995) discussed above. These authors predict that such alterations in phospholipid composition of the membrane are responsible for biochemical instability of RBC in Pb-exposed workers. Zimmermann et al. (1993) investigated the potential of such membrane lipid alterations to cause resistance to oxidation. These investigators induced hyperlipidemia by treating Pb-exposed Wistar rats with triton and observed an increase in erythrocyte choline phospholipid levels, together with a significant decrease in membrane lipid resistance to oxidation. They postulated that such a decrease in resistance might cause RBC fragility and ultimate destruction, leading to anemic conditions. It has been also reported that exposure to Pb may also increase the levels of fatty acids, e.g., arachidonic acid, in the RBC membrane in humans exposed to Pb (Osterode and Ulberth, 2000). Based on the negative correlation between serum calcium and increased arachidonic acid content, these authors postulated that Pb ions might have substituted for calcium in the activation of phospholipase enzymes, leading to increased synthesis of arachidonic acid. The fact that these biochemical and molecular changes were reported at somewhat higher blood Pb levels (70 μg/dL) probably does not undermine these observations made from the RBCs of humans exposed to Pb over a period of time and enhances our understanding of the several molecular facets that may play a role in the altered erythrocyte mobility. Suwalsky et al. (2003) investigated the interaction of Pb with the RBC membrane, utilizing intact as well as isolated unsealed RBC membrane models (representing phospholipids present in the inner and outer layers of the membrane). Electron microscopy, fluorescence spectroscopy, and X-ray diffraction analyses of these models by Suwalsky et al. (2003) indicated that Pb particles adhere to both external and internal surfaces of the membrane. Pb ions also have been found to disturb the lamellar organization by causing considerable molecular disorder within lipid layers.

Recently, it has been shown that osmotic shock, oxidative stress, and/or energy depletion activate Ca^{2+}-sensitive erythrocyte scramblase, leading to the exposure of phosphotidylserine at the cell surface. This exposure of phosphotidylserine had been implicated in the phagocytosis of RBC by macrophages that can be measured by annexin binding, as determined by fluorescence activated cell sorting analysis. Kempe et al. (2005) carried out experiments to investigate whether anemic conditions reported in Pb intoxication are the result of the decreased life span of RBCs due to the above-mentioned mechanisms. These investigators reported that when human RBCs were exposed to Pb nitrate (above 0.3 μM), it caused a significant increase in Pb annexin

binding, indicative of phosphotidylserine exposure. Using inhibitors for Ca^{2+}-sensitive potassium channels and whole cell patch clamp experiments, they concluded that Pb exposure increased activation of potassium channels, leading to shrinkage of cells and also activation of scramblase, resulting in the exposure of phosphotidylserine on the cell membrane surface. These authors further postulated that this exposure of phosphotidylserine on the membrane might have led to them being engulfed by macrophages and the ultimately decreased life span of RBCs in Pb intoxication.

Membrane Proteins

Earlier, Fukumoto et al. (1983) reported the differential profile for RBC-membrane polypeptides determined by SDS-PAGE analysis. These investigators found decreased levels of polypeptides in band 3 and increases in the levels of four other bands (i.e., bands 2, 4, 6, and 7) in the RBCs of human workers exposed to Pb. From these observations, they postulated that such Pb-induced alteration in RBC membrane proteins may lead to membrane permeability changes. Apostoli et al. (1988) also observed similar changes in RBC membrane polypeptides in Pb-exposed workers and suggested that band 3 may represent an anion channel protein; they also found that these changes occurred at blood Pb levels >50 µg/100 mL.

Lead exposure has been known to increase the amount of membrane-bound protein kinase C in rat brain, endothelial, and glial cells. Belloni-Olivi et al. (1996) reported an increased phosphorylation of RBC membrane proteins on Pb exposure. When human RBCs were incubated with Pb acetate (>100 nM) for 60 min, increased phosphorylation of membrane cytoskeletal proteins (120, 80, 52 and 45 kDa) was found, accompanied by increased protein kinase C activity. Membrane proteins were not phosphorylated when treated with protein kinase C inhibitors. Calcium and diacylglycerol were found not to be involved in this process. The authors suggested that this activation of protein kinase was a direct interaction of the enzyme protein with Pb. Slobozhanina et al. (2005) reported that incubation of human RBCs with Pb acetate (1 to 10 µM for 3 h) caused differential binding of fluorescent probes to the membrane, suggesting alterations in the physicochemical state of the membrane proteins and lipids. Based on these observations, the authors postulated that such alterations in membrane molecular composition may influence the activity of membrane enzymes and the functioning of receptors

and channels present on the membrane. These and related studies are summarized in Annex Table AX5-2.1.

5.2.3 Effects of Lead on Erythrocyte Heme Metabolism

Enzyme studies of the heme pathway have shown that Pb is an inhibitor of several enzymes involved in heme synthesis, including 5-aminolevulinic acid dehydratase (ALAD), coproporphyrinogen oxidase, and ferro chelatase (see Figure 5-1 for a schematic representation of heme biosynthesis). ALAD is a cytoplasmic enzyme that catalyzes the second, rate-limiting step of the heme biosynthesis pathway; that is, ALAD catalyzes formation of porphobilinogen through the conjugation of two molecules of δ-aminolevulinic acid. ALAD is a Zn-dependent enzyme, and thiol groups are essential for its activity (Bernard and Lauwerys, 1987). Decreased erythrocyte ALAD is the most sensitive indicator of human Pb exposure, to the extent that measurement of ALAD activity reflects well Pb levels in the blood. Similarly, erythrocyte ALAD activity measurements have been used to assess Pb toxicity in other species.

Erythrocyte ALAD

Terayama et al. (1986) reported decreased ALAD activity in rat RBCs at blood Pb levels of 10 µg/dL. Scheuhammer (1987) studied the usefulness of the ALAD ratio (activated/nonactivated enzyme activity) to study Pb effects in avian RBCs. The ALAD activity ratio is a sensitive, dose-responsive measure of Pb exposure regardless of the mode of Pb administration. For example, dietary Pb concentrations as low as 5 ppm (dry weight) can be estimated through the use of the ALAD enzyme activity ratio method. A highly significant positive correlation was observed between dietary Pb concentration over the 5 to 100 ppm range and the ALAD activity ratio. The author concluded that RBC ALAD ratio may be a useful method for estimating average dietary concentrations of Pb over an environmentally relevant range, in situations where diet is the major source of exposure to Pb or where accurate estimations of dietary Pb are not possible. Redig et al. (1991) reported heme synthetic pathway alterations upon chronic exposure (3 or 11 weeks) to Pb in red-tailed hawks. This treatment resulted in a severe decrease in RBC ALAD activity, which did not return to normal levels until 5 weeks after termination of Pb treatment. Lead exposure also decreased ALAD activity in the bone marrow and in the liver but did not alter aminolevulinic acid synthase activity.

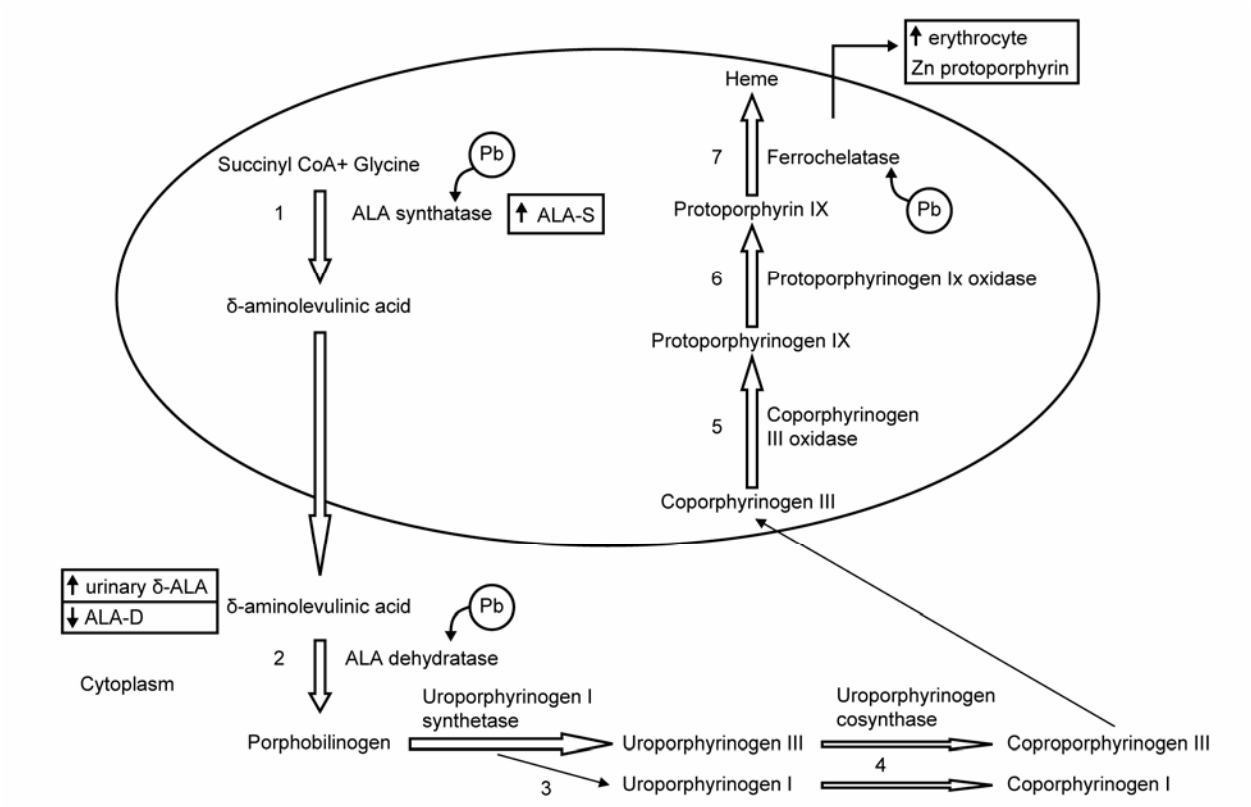

Figure 5-1. Schematic presentation of the enzymatic steps involved in heme synthesis pathway. Potential lead (Pb) interacting sites are indicated by curved arrows (⇧ increased, ⇩decreased)

Source: Modified from U.S. EPA (1986a).

Dorward and Yagminas (1994), using comparative enzyme kinetic analysis of ALAD in Pb-exposed female cynomolgus monkeys and human erythrocyte ALAD, found similar inhibition profiles and concluded that ALAD could be a useful model for measuring the biological response in monkeys. Santos et al. (1999) reported that rat RBC heme biosynthesis was affected by either Pb treatment alone or Pb in combination with ethanol, due to the inhibition of ALAD activity.

Analysis of blood ALAD activity had been used as a powerful clinical biomarker in evaluating Pb toxicity in occupational exposure. Fontanellas et al. (2002) further suggested that this enzyme assay be used in identifying even subclinical Pb poisoning in chronic renal failure (see Section 5.7 for details).

Other Heme Metabolism Enzymes

Taketani et al. (1985) studied the heme synthesizing activity of ferric ion using purified ferrochelatase from rat liver mitochondria and reported that Pb reduced NAD(P)H-dependent heme synthesis by 50% at 10^{-5} M, but that it had no effect when ferrous ion was used as the substrate. Based on these results, the authors concluded that heme synthesis from ferric ion was more susceptible to Pb than the ferrous ion. These studies also revealed that the NAD(P)H oxidizing system reduces ferric ion to ferrous ion, which in turn was used for heme synthesis by ferrochelatase.

The effect of various metals, including Pb, on RBC porphobilinogen synthase (PBG-S) was studied using human RBC hemolysate. Farant and Wigfield (1987) reported that the effect on the enzyme depends on the affinity of the metal for thiol groups at its active sites. Additional studies carried out by the same group utilizing rabbit erythrocyte PBG-S indicated that Pb acts as a potent effector of this enzyme both in vitro and in vivo (Farant and Wigfield, 1990). Human RBC porphobilinogen synthetase activity was found to be inhibited by Pb, whereas Zn ions activated this enzyme (Simons, 1995). Another enzyme involved in the heme synthetic pathway, porphobilinogen deaminase, was inhibited in human RBC by Pb nitrate (100 mM) in in vitro studies, but had no effect in vivo (Tomokuni and Ichiba, 1990). Rossi et al. (1992) reported no inhibition of coproporphyrinogen oxidase activity in human lymphocytes on exposure to Pb. Heme synthesis can also be affected in Pb intoxication by interference with Fe transport into reticulocytes. Using a rabbit reticulocyte model, Qian and Morgan (1990) reported that inhibitory effects of Pb on transferrin endocytosis and iron transport across the membrane may also contribute to altered heme metabolism in RBCs. These and other related studies are summarized in Annex Tables AX5-2.2 and AX5-2.3.

5.2.4 Effects of Lead on Other Hematological Parameters

The RBC pyrimidine 5-nucleotidase (P5N) catalysis of the hydrolytic dephosphorylation of pyrimidine 5-monophosphates is sensitive to inhibition by Pb. Tomokuni et al. (1989) evaluated the activity of RBC and bone marrow 5-nucleotidase (P5N) and RBC ALAD in mice exposed to drinking water Pb (200 to 500 ppm) for 14 or 30 days. These authors reported that Pb exposure decreased both P5N and ALAD activities in erythrocytes. Additional studies from this group, using a similar exposure regimen, indicated no change in levels of urinary coporphyrins.

Lead exposure (4 mg/kg and 6 mg/Kg body wt/30 days) in splenectomized rats was found to cause depletion of RBC Hb content, to increase numbers of reticulocytes in peripheral blood, and to increase urinary delta aminolevulinic acid excretion (Gautam and Chowdhury, 1987). These authors further reported that the increased number of reticulocytes found in the blood may be due to induced acceleration of the erythropoeitic cell series. Redig et al. (1991) reported biphasic effects of Pb on hematological parameters from their chronic exposure studies in red-tailed hawks over 3 or 11 weeks. These authors observed a rapid and relatively brief increase in RBC free protoporphyrin and a slower, but more prolonged, increase in its Zn complex with 3-week exposure to Pb (0.82 mg/kg body wt). On the other hand, exposure to a higher dose of Pb (1.64 mg/kg body wt) for a longer duration (11 weeks) resulted in a decrease in the Hct and Hb. Panemangalore and Bebe (1996) reported that Zn deficiency increased the Pb-induced accumulation of porphyrin in RBCs to a lesser extent compared to its accumulation in the liver in weaning rats.

The effects of Pb on RBC number and other Hct parameters appear to be dose dependent. Iavicoli et al. (2003) investigated these effects by feeding mice with eight different doses of Pb below (0.6 to <2.0 μg/dL) and above (>2.0 to13 μg/dL) normal background levels. These authors reported that mice receiving below normal background levels of dietary Pb displayed enhanced RBC counts and increased Hb and Hct values, whereas a marked decrease in RBC number occurred when blood Pb levels approached 10 μg/dL. Sivaprasad et al. (2003) also reported significant reductions in RBC Hb content and Hct on Pb exposure (0.02% Pb acetate in drinking water for 5 weeks). Toplan et al. (2004) observed significant decreases in RBC Hb content and Hct and increases in blood viscosity in Wistar rats after 5-week exposure to Pb. Studies cited above are summarized in Annex Table AX5-2.4.

5.2.5 Effects of Lead on Erythrocyte Enzymes

The toxic effects of Pb on RBCs result from its complexation with the sulfhydryl, carboxyl, and imidazole groups of proteins, particularly enzymes, by competitive binding of Pb^{2+} with Zn^{2+} or Mg^{2+} in metalloenzymes. This binding of Pb to enzyme proteins can inhibit enzymes involved in the glycolytic and pentose phosphate pathway, both of which are sources of energy compounds and intermediates of purine conversion, thus causing a disruption of energy metabolism. Along with these changes, Pb-induced changes in the membrane integrity, as

discussed earlier (Section 5.2.1), may also affect the enzymes' associated ion channels and other transport mechanisms.

Energy Metabolism

Erythrocytes generate high-energy ATP by anerobic glycolysis and cycle oxidized and reduced nicotinamide adenine dinucleotide phosphate (NADP) by the aerobic pentose phosphate pathway. Anemic conditions associated with Pb poisoning, along with the inhibitory effects of Pb on heme synthesis, may result in increased RBC destruction due to the inhibitory effects of Pb on the activities of the enzyme, pyramidine 5-nucleotidase (P5N). Deficiency of this enzyme is characterized by intracellular accumulation of pyramidine-containing nucleotides, leading to hemolysis. Inhibition of this enzyme, along with perturbations in heme metabolism, creates imbalances in the energy currency of the erythrocyte. Perturbations in energy metabolism can be followed by changes in the concentration of purine nucleotides. In erythrocytes, these compounds cannot be synthesized de novo; they can only be reconstructed from preexisting free purine bases on nucleosides through salvage type reactions. The cell energy content can be measured by adenylate (ATP + ADP + AMP) and guanylate (GTP + GDP + GMP) nucleotides, and by their sum total. The concentrations of nucleoside monophosphates increase in cases of cell energy deficit, but they quickly degrade to nucleosides and bases.

Cook et al. (1987) compared P5N and deoxypyramidine-5-nucleotidase levels in the RBC of Pb-exposed workers and matched controls and reported significantly lower levels of P5N in Pb-exposed workers. Konantakieti et al. (1986) reported similar observations in neonatal rat RBCs. These authors further indicated that the low levels of nucleotides were due to inhibition of P5N activity by Pb, as the depression in enzyme activity was correlated with blood Pb levels. This was further validated by in vitro inhibition of P5N in a dose-dependent manner. Tomokuni and Ichiba (1988) found similar results with human RBCs both in vitro and in vivo. They reported activation of Pb-exposed human RBCs. Antonowicz et al. (1990) observed significantly higher levels of glycolytic enzymes and increased production of lactic acid and 2,3-diphospho glycerol, when human RBCs were incubated with Pb. Based on their observations, these authors suggested that Pb exposure may result in anaerobic glycolysis activation in human RBCs.
In contrast, Grabowska and Guminska (1996) reported that Pb exposure diminished the ATP levels in human RBCs by inhibiting aerobic glycolysis.

Erythrocyte energy metabolism in workers exposed to heavy metals, but without clinical manifestations of toxicity, was found to intensify and become more pronounced when they were occupationally exposed to Pb. Nikolova and Kavaldzhieva (1991) measured the exposed workers and reported higher ratios of ATP/ADP in Pb-exposed workers. Because the RBC energy pool is perturbed due to Pb exposure, Morita et al. (1997) evaluated the effect of Pb on NAD synthetase and reported an apparent dose-dependent decrease in NAD synthetase activity in the erythrocytes of Pb exposed workers.

Baranowska-Bosiacka and Hlynczak (2003) evaluated Pb effects on distribution profiles of adenine, guanine nucleotide pools, and their degradation products in human umbilical cord RBCs. In vitro exposures (Pb acetate; 100 to 200 µg/dL) equivalent to Pb exposure for 20 h were found to significantly lower the levels of nucleotide pools, including NAD and NADP, accompanied by a significant increase in purine degradation products (adenosine, guanosine, inosine, and hypoxanthine). Associated morphological RBC alterations were also observed, with marked significant increases in stomatocytes, spherocytes, and echinocytes. These investigators also observed similar alterations in the nucleotide pools in Wistar rat RBCs with short-term exposure to Pb (Baranowska-Bosiacka and Hlynczak, 2004). Based on these observations, the authors postulated that decreases in NAD and NADP concentrations in RBCs may be a good indicator of Pb-induced disturbance in the energy process and can serve as a useful marker for chronic Pb exposure. If NAD synthetase activity had been measured in these studies, it might have provided experimental support for the observation of inhibition of NAD synthetase reported by Morita et al. (1997).

Other Enzymes

Lead-induced efflux of K^+ from human RBCs had been recognized as being due to the ability of Pb to selectively increase the membrane permeability for this cation. Studying the efflux of ^{86}Rb using inside-out RBC vesicles, Alvarez et al. (1986) demonstrated that Pb promoted the selective efflux of K^+ ions by altering the sensitivity of Ca^{2+} binding site on the membrane either by direct binding or by altering Mg^{2+}-mediated modulation. Fehlau et al. (1989) indicated that this modulation of the Ca^{2+}-activated K^+ channel in human RBCs coincides with the activation of RBC membrane-bound oxidoreductase. These authors suggested that,

even though these two are independent events, the oxidoreductase enzyme activity may influence K channel gating.

Earlier studies by Mas-Oliva (1989) on the potential effects of Pb on the RBC membrane (using RBC ghosts) indicated that Pb has inhibitory effects on Ca^{2+}-Mg^{2+}-ATPase. Further investigations on the role of calmodulin in the inhibition of Ca^{2+}-Mg^{2+}-ATPase indicated that the inhibitory activity on the enzyme may be due either to the effect of Pb on sulfhydryl groups on the enzyme or by direct binding to calmodulin.

Jehan and Motlag (1995) reported that when albino rats were administered Pb i.p (5 or 20 mg/kg body wt) for 14 consecutive days either alone or in combination with Cu (2 mg/kg body wt) or zinc (5 mg/kg body wt), there were severe decreases in RBC membrane enzyme, acetylcholine esterase (AChE), NADH dehydrogenase, and Na^{+}-K^{+} ATPase levels along with decreases in phospholipid content, hexose, and hexosamine. Of the combined metal treatment exposure regimens, Zn was found to considerably reduce such changes. Grabowska and Guminska (1996) assayed three ATPase activities (i.e., Na^{+}-K^{+} ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase) in human RBC in vitro and reported RBC Na^{+}-K^{+} ATPase to be the only enzyme inhibited by Pb, while Ca^{2+} or Mg^{2+}ATPases were not sensitive to Pb. On the other hand, Sivaprasad et al. (2003) observed Pb-induced reductions in RBC activities for all three of those types of ATPase activities.

Two reports by Calderón-Salinas et al. (1999a,b) indicated Pb effects on Ca transport in human RBC. Initial studies by this group indicated that Pb and Ca are capable of inhibiting the passive transport of other metals in a noncompetitive way. Inhibition studies using N-ethyl-maleimide indicated that Pb and Ca share the same permeability pathway in human RBCs and that this transport system is electrogenic (Calderón-Salinas et al., 1999a). Additional studies by the same group reported that Pb is capable of inhibiting Ca efflux by inhibiting Ca-ATPase (Calderón-Salinas et al., 1999b). These authors further suggested that under physiological conditions, Pb, via Ca^{2+}-ATPase, alters Ca influx, while chronic Pb intoxication inhibits Ca efflux by altering RBC Ca homeostasis. Silkin et al. (2001) reported Pb-induced activation of K channels in the RBCs of the teleost fish *S. porcus*. Exposure of teleost fish RBCs to 1 to 2 μM Pb led to a minor loss in cellular K^{+}; but, at 20 to 50 μM Pb, about 70% of cellular K^{+} was lost. Based on their observations of Pb-induced K^{+} efflux from RBCs under competitive and inhibitory regimens, these authors suggested that Pb activates RBC K^{+} channels.

Eder et al. (1990) and Loipführer et al. (1993) investigated activity levels of Ca^{2+}-ATPase and Ca accumulation, respectively, in Pb-depleted rat RBCs. No alteration in Ca^{2+}-ATPase activity or Ca accumulation was observed in the P0 generation (Eder et al., 1990). On the other hand, significant reduction in Ca-ATPase activity was observed in the F1 generation. It was suggested that Pb-induced alterations in the metabolism of phosphoproteins and glycoproteins result from Pb depletion and may be responsible for the reduced enzyme activity. Both of the groups postulated that the decreased MCV observed in Pb depleted rat RBCs could be due to reduced Ca^{2+}-ATPase activity in the RBCs. These and other related studies are summarized in Annex Tables AX5-2.5 and 5-2.6.

5.2.6 Erythrocyte Lipid Peroxidation and Antioxidant Defense

Although several mechanisms have been proposed to explain Pb toxicity, no mechanisms have been defined explicitly. Recent literature on Pb toxicity suggests oxidative stress as one of the important mechanisms for toxic effects of Pb in various organs. Because RBCs accumulate major amounts of Pb compared to other tissues, oxidative stress may also result in the accentuation of lipid peroxidation, with concomitant inhibition of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, GSH peroxidase, GSH reductase, and simultaneous increases in oxidized GSH (GSSG) and reduced GSH/GSSG ratios. Below, Pb-induced lipid peroxidation and the mitigating effects of experimental chelation therapy are discussed with relevance to each tissue or organ within this chapter. The discussion focuses on the available literature with reference to studies on erythrocytes.

Patra and Swarup (2000) reported significant changes in RBC lipid peroxide levels and anti oxidant defense (SOD and catalase) levels in RBC hemolysates from male calves exposed to Pb (7.5 mg/kg body wt for 28 days). These authors suggested the potential role for increased peroxide levels in Pb-induced alterations in RBCs. Mousa et al. (2002) investigated the levels of various antioxidant enzymes, thiols, lipid peroxide in erythrocytes, and total thiol status of plasma in goats exposed to Pb (Pb acetate, 5.46 mg/kg body wt for 2 weeks). These authors reported that all the parameters referred to above were significantly increased in RBCs by day 7 and receded to normal levels by day 14, while peroxides remained significantly increased even by day 14. Based on these observations, it was suggested that Pb-induced lipid peroxide generation in RBCs appears to be a continuous process and can lead to persistent oxidative stress

in RBCs with chronic exposure. The effects of chelative agents on RBC lipid peroxidation are summarized in Annex Table AX5-2.7.

5.2.7 Summary

- The 1986 Lead AQCD reported that the activity of ALAD appeared to be inversely correlated to blood Pb values and was found inhibited in several tissues. Human studies reviewed in 1986 Lead AQCD also indicated that occupational exposure to Pb results in decreased RBC survival along with alterations in RBC membrane integrity and energetics.

- More recent studies reviewed in this AQCD indicate that the transport of Pb across the RBC membrane is energy-independent, carrier-mediated, and that the uptake of Pb is mediated by an anion exchanger through a vanadate-sensitive pathway.

- Lead intoxication interferes with RBC survival and alters RBC mobility. Hematological parameters, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), are also significantly decreased upon exposure to Pb. These changes are accompanied by decreased membrane sialic acid content.

- Morphological analyses using electron paramagnetic resonance imaging and spin labeling techniques indicate that changes occur in RBC morphology upon Pb exposure.

- Lead-induced RBC membrane lamellar organization and decreases in membrane lipid resistance to oxidation in rats appear to be mediated by perturbations in RBC membrane lipid profiles. Similarly, Pb-induced altered phosphorylation profiles of RBC membrane proteins have been reported.

- Erythrocyte ALAD activity ratio (ratio of activated/non activated enzyme activity) has been shown to be a sensitive, dose-responsive measure of Pb exposure, regardless of the mode of administration of Pb. Competitive enzyme kinetic analyses in RBCs from both human and Cynomolgus monkeys indicated similar inhibition profiles by Pb.

- Consistent observation of Pb-mediated inhibition of pyramidine 5′-nucleotidase (P5N) suggests this enzyme as a potential biomarker for Pb exposure.

- Significant reductions in levels of nucleotide pools (e.g., NAD and NADP) accompanied by significant increase in purine degradation products have been implicated in the Pb-induced altered energetics of RBCs.

- Lead-induced increased permeability for K^+ in RBCs appears to be due to the selective efflux of K^+ ions on the RBC membrane due to altered sensitivity of the Ca^{2+}-binding site on the membrane. Erythrocyte Na^+-K^+ ATPase appears to be more sensitive to Pb-induced inhibition than Ca^{2+}-Mg^{2+} ATPase.

The newly available (since 1986) scientific evidence presented in this section clearly demonstrates deleterious Pb effects on erythrocyte cell morphology and function, as well as Pb uptake and alterations in certain enzymes involved in heme synthetic pathways. However, some of the interesting and important conclusions are derived mainly from in vitro studies, often using short time incubations. It would be useful to substantiate such findings further by more systematic studies employing meaningful experimental designs for in vivo evaluation of laboratory animal models.

5.3 NEUROLOGIC/NEUROBEHAVIORAL EFFECTS OF LEAD

5.3.1 Introduction

Since the initial description of Pb encephalopathy in the developing rat in the mid-1960s (Pentschew and Garro, 1966), a continuing research focus has been on defining the extent of CNS involvement at subencephalopathic, environmentally relevant levels of exposure. These efforts have primarily addressed the developing animal, consistent with the primary public health concerns for neurotoxicity from Pb during this period. While significant research advances have been made in animal studies over the last four decades, relating these findings to neurotoxicity in children has been challenging and difficult. The barriers to greater progress have primarily been due to Pb's multiple toxic mechanisms of action in brain tissue, which encompass variable, overlapping, and, at times, opposing dose-effect relationships. The goal of this section is to bring greater clarity to the current state of knowledge.

Discussions of the biologic effects of Pb in the 1986 Lead AQCD focused on general questions relating to (1) the internal exposure levels, as measured by blood Pb concentrations, at which neurotoxic effects occur; (2) the persistence or reversibility of these effects; and (3) the populations that are especially sensitive to the neurotoxic effects of Pb. The state of knowledge at publication of the 1986 AQCD provided answers for these questions as follows.

At very high levels of exposure producing blood Pb levels of 100 to 120 µg/dL in adults and 80 to 100 µg/dL in children, serious neurotoxic effects occur, including acute Pb encephalopathy that can progress to convulsions, coma, and sudden death. Less severe exposures creating blood Pb levels of 40 to 60 µg/dL produce both central and peripheral nerve dysfunction, including slowed nerve conduction velocity and overt signs and symptoms of

neurotoxicity. Decrements in IQ are observed in children with blood Pb concentrations of 30 to 50 μg/dL, with some studies showing effects at lower blood Pb levels. Neurobehavioral effects are observed in rats and monkeys at levels <20 μg/dL.

(1) Human studies provide little information on the persistence of effects. Animal studies show that alterations in neurobehavioral function can persist long after lead exposure has stopped and PbB levels have returned to normal. Persistent learning deficits occur in both rats and monkeys, consistent with morphologic, electrophysiologic, and biochemical endpoints that indicate lasting changes in synaptogenesis, dendritic development, myelin and fiber track formation, ionic mechanisms of neurotransmission, and energy metabolism.

(2) Animal studies show that the order of susceptibility of neurotoxic effects of lead is young > adults and female > male.

At the time of publication of the 1986 AQCD, one line of evidence concerned the effects of acute exposure to Pb^{2+} in vitro on voltage-sensitive Ca^{2+} channel function in the nerve cell membrane, developed to a great extent by Cooper and co-workers (Kober and Cooper, 1976; Cooper and Manalis, 1984; Suszkiw et al., 1984).

In the ensuing two decades, the Pb neurotoxicity literature has reflected an increased focus on cognitive-related mechanisms and the refinement of approaches and methodologies. Exposure-induced alterations at glutamatergic synapses have received considerable attention. Synaptic plasticity models (e.g., long-term potentiation [LTP]) came into use in the 1990s for Pb studies in laboratories around the world. Behavioral paradigms, refined to more consistently discriminate Pb effects, aided in identifying optimal testing conditions and developmental periods for exposure. These advances have led to a clearer understanding of the likely mechanisms underlying Pb-induced cognitive impairments in exposed children.

The evidence for Pb neurotoxicity reviewed in this section is organized largely according to scientific discipline: neurochemical alterations involving glutamatergic, cholinergic, and dopaminergic function; mechanisms defined by neurophysiologic approaches; changes in auditory and visual function; identification of altered components of behavioral function; induced alterations in cellular morphology; and findings on cellular disposition of Pb. This type of organization permits a more focused analysis of an extensive and broad literature. In each section below, a brief description of work previously described in the 1986 AQCD introduces each section. An integrative synthesis of the health effects of Pb exposure based on toxicologic and epidemiologic findings is presented in Chapter 8.

5.3.2 Neurochemical Alterations Resulting from Lead Exposure

Earlier work demonstrated that Pb interfered with chemically mediated synaptic transmission, probably due to its resemblance to endogenous divalent cations. At that time, a selective vulnerability of any particular neurotransmitter system to the effects of the metal was not apparent. Some generalizations made in the 1986 AQCD regarding neurochemical effects at blood Pb levels of ~50 to ~90 µg/dL were as follows.

(1) Synthesis, turnover, and uptake of dopamine and norepinephrine are depressed in the striatum and elevated in the midbrain, frontal cortex, and nucleus accumbens. These changes were paralleled by concomitant increases in dopamine receptor binding in the striatum and decreases in dopamine receptor binding in the nucleus accumbens, possibly involving a specific subset (D2) of dopamine receptors.

(2) The findings for pathways utilizing γ-aminobutyric acid (GABA) showed similar parallels. Increases in GABA synthesis in the striatum are coupled with decreases in GABA receptor binding in that region, while the converse holds true for the cerebellum. In these cases, cyclic GMP activity mirrors the apparent changes in receptor function.

The following areas of investigation discussed below have been accorded notable attention in the Pb neurotoxicity field over the last 20 years, as reflected by the number of papers published and number of investigators with these research foci.

Lead and Neurotransmitter Release Processes

By the mid-1980s, it was evident that acute exposure to Pb^{2+} in vitro reduced the magnitude of depolarization-induced transmitter release, apparently by inhibiting Ca^{2+} influx into the nerve ending through voltage-sensitive Ca^{2+} channels (Kober and Cooper, 1976; Cooper and Manalis, 1984; Suszkiw et al., 1984). Since then, several investigators utilizing various preparations (Shao and Suszkiw, 1991 [cortical synaptosomes]; Tomsig and Suszkiw, 1993 [bovine chromaffin cells]; Braga et al., 1999a,b [cultured hippocampal cells]; Westerink and Vijverberg, 2002 [PC12 cells]) have demonstrated that in the absence of Ca^{2+}, Pb^{2+} exhibits Ca^{2+}-mimetic properties in stimulating exocytosis and is substantially more potent in doing so. That is, in the absence of Ca^{2+} and depolarization, nanomolar concentrations of Pb^{2+} alone stimulate transmitter release. Many investigators have proposed that this action, in conjunction with the ability of Pb^{2+} to suppress evoked release of neurotransmitters, produces a higher noise level in synaptic transmission in Pb-exposed animals.

Lead has been shown to diminish stimulated transmitter release in intact chronically exposed animals with blood Pb values in the range of ~20 to 40 µg/dL via intracerebral microdialysis (Kala and Jadhav, 1995; Lasley and Gilbert, 1996; Lasley et al., 1999). More recently, Lasley and Gilbert (2002) used Ca^{2+}-free perfusate containing a Ca^{2+} channel antagonist for microdialysis to identify the Ca^{2+}-independent component of neurotransmitter release. Under these conditions, high K^{+}-stimulated release of glutamate and GABA was *elevated* in chronic Pb-exposed animals, suggesting a Pb^{2+}-induced enhancement of evoked release at higher exposure levels. It was concluded that this pattern of results indicated the presence of two actions of Pb on transmitter release in vivo: (1) a more potent suppression of stimulated release seen at lower exposure levels (associated with blood Pb values of 27 to 62 µg/dL) combined with (2) Ca^{2+}-mimetic actions that independently induce the exocytosis seen at higher exposure levels (associated with blood Pb values of ≥62 µg/dL). Together, these two actions produce a biphasic dose-effect relationship (see Figure 5-2). Thus, there is good correspondence between findings of in vitro and in vivo studies with respect to the actions of Pb on transmitter release.

Lead and Glutamatergic NMDA Receptors

Because of the established importance of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor in synaptic plasticity and learning, these receptors have been a focus of intense interest in Pb neurotoxicity for the last 15 years. Using whole cell and single channel patch clamp methodologies, Alkondon et al. (1990) were the first to report that Pb^{2+} inhibited the function of the NMDA receptor channel complex. Guilarte and Miceli (1992) reported similar findings using nominal Pb^{2+} concentrations and receptor binding techniques and drew parallels between Zn^{2+}-, Ca^{2+}-, and Pb^{2+}-induced inhibition of the channel. However, Lasley and Gilbert (1999), using free Pb^{2+} ion concentrations and radioligand binding, demonstrated that despite similarities to the actions of Zn^{2+}, Pb^{2+} did not inhibit the NMDA receptor channel complex by binding to the Zn^{2+} allosteric site. Furthermore, these workers indicated that the Pb^{2+} IC_{50} of 0.55 µM for inhibition of the channel complex was likely about two orders of magnitude greater than the extracellular fluid concentrations of Pb^{2+} associated with environmentally relevant exposure. This does not imply that NMDA receptor function does not change after Pb exposure, but it strongly suggests that the alterations are not based on a direct Pb^{2+} action.

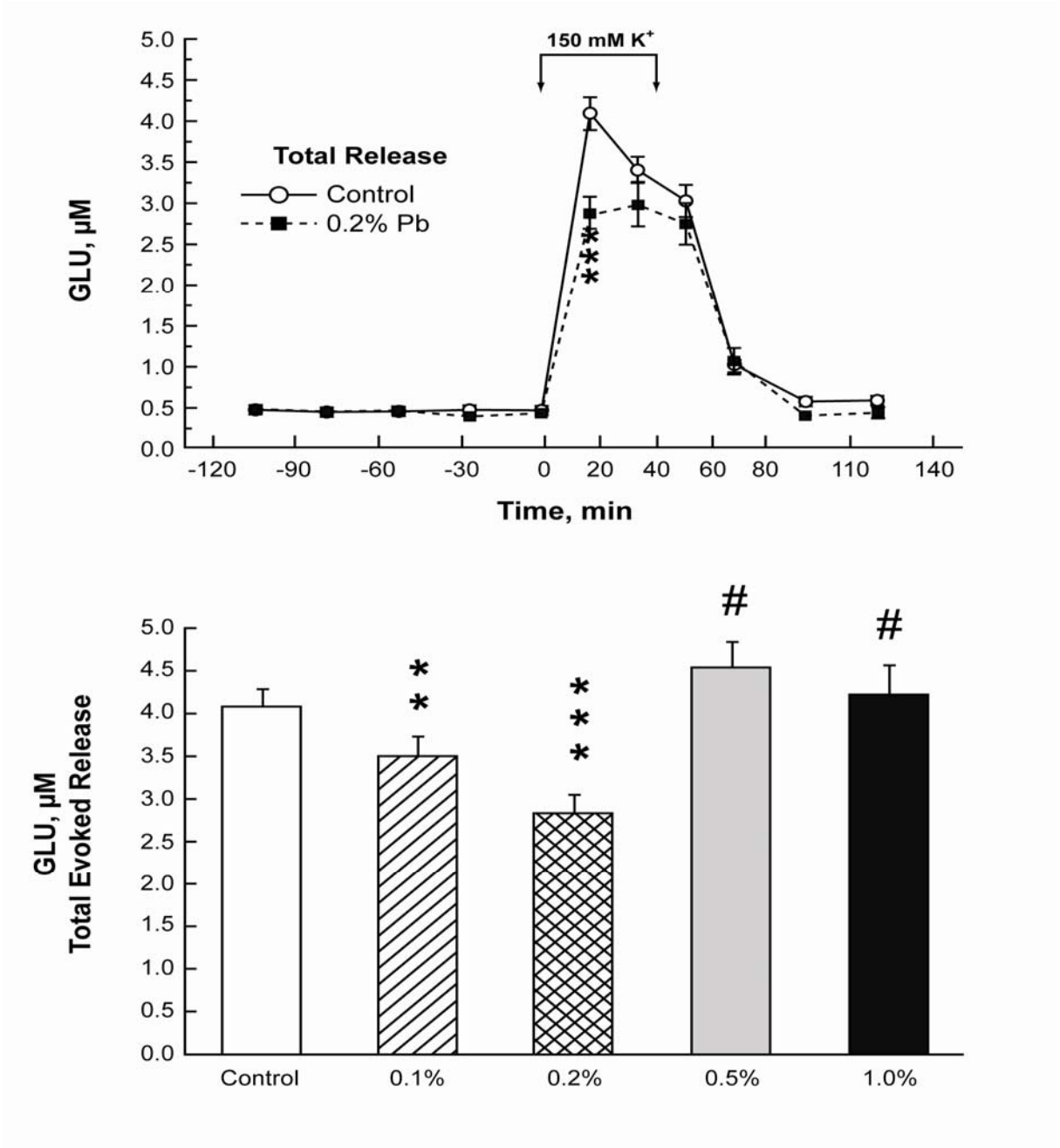

Figure 5-2. Time course and magnitude of response of extracellular GLU concentration as a result of chronic lead exposure.

*** $p < 0.001$; ** $p < 0.01$ relative to the GLU concentration in control animals;
[#]$p < 0.0001$ relative to the GLU concentration in the 0.2% Pb group.

Source: Lasley and Gilbert (2002).

Unfortunately, a consensus on the effects of chronic Pb exposure on NMDA receptor expression and function has not been achieved. Extensive effort has been invested to assess NMDA receptor subunit mRNA and protein expression in exposed animals with blood Pb values in the range of 25 to 45 µg/dL (Guilarte and McGlothan, 1998; Nihei and Guilarte, 1999; Guilarte et al., 2000; Nihei et al., 2000; Toscano et al., 2002; Guilarte and McGlothan, 2003), but consistent findings have not emerged. A possible exception was the work of Nihei et al. (2000, 25 to 32 µg/dL) who found decreases in hippocampal NR1 subunit mRNA and protein expression associated with animals that exhibited deficits in LTP and spatial learning after chronic exposure. Correlations of this type with functional measures are valuable in validating biochemical observations. However, it should also be noted that such correlations do not confirm a direct relationship between LTP or the behavior and the NMDA receptor subunit changes.

While exposure-induced alterations of NMDA receptor binding have been observed in multiple laboratories, there has been no uniform agreement as to the direction of change. Upregulation of NMDA receptor density has been observed in rats continuously exposed throughout development with blood Pb values in the range of 39 to 62 µg/dL (Ma et al., 1997; Lasley et al., 2001), but receptor downregulation has also been reported when exposure was begun immediately postweaning and blood Pb levels achieved 16 to 28 µg/dL (Cory-Slechta et al., 1997a). The results of behavioral investigations are most parsimoniously explained by increases in NMDA receptor density. Cohn and Cory-Slechta (1993, 1994a), using a repeated learning component of a multiple reinforcement schedule, observed enhanced performance sensitivity to exogenous NMDA administration and diminished sensitivity to MK-801, an NMDA receptor antagonist, in exposed animals with blood Pb values of 25 to 74 µg/dL. The same findings resulted when a drug discrimination paradigm was utilized (Cory-Slechta, 1995; Cory-Slechta et al., 1996b): enhanced sensitivity to NMDA and reduced sensitivity to MK-801 in Pb-exposed groups in the presence of blood Pb values in the range of 13 to 36 µg/dL. A decreased sensitivity to MK-801 can result from either increased numbers of NMDA receptors or a diminished access of the antagonist to its binding site in the ion channel. Thus, all these behavioral observations may be accounted for by Pb-induced increases in NMDA receptor density resulting in increased sensitivity to agonists coupled with decreased sensitivity to antagonists. That is, the functional measures suggest that an NMDA receptor upregulation

occurs. This interpretation should not preclude the possibility that experimental outcomes can change significantly in the presence of apparently small modifications in exposure parameters.

Pb^{2+}-Ca^{2+} Interactions

At the time of publication of the 1986 AQCD/Addendum, one of the most reproducible lines of evidence concerned the effects of acute exposure to Pb^{2+} in vitro on voltage-sensitive Ca^{2+} channel function in the nerve cell membrane, developed to a great extent by Cooper and co-workers (Kober and Cooper, 1976; Cooper and Manalis, 1984; Suszkiw et al., 1984). Using neuromuscular endplate or synaptosomal preparations, these studies demonstrated that Pb^{2+} interfered with Ca^{2+} influx through voltage-sensitive channels. Subsequent work has replicated and extended these findings (e.g., Tomsig and Suszkiw, 1993; Westerink and Vijverberg, 2002), and has demonstrated that Pb^{2+} exhibits Ca^{2+}-mimetic properties in stimulating transmitter exocytosis. While acute exposure in vitro has been assumed to bear little resemblance to environmentally relevant routes and magnitudes of exposure, recent findings nonetheless suggest that inhibition of Ca^{2+} influx through voltage-sensitive Ca^{2+} channels and the Ca^{2+}-mimetic properties of Pb^{2+} are important neurotoxic mechanisms in intact animals across a range of chronic exposure levels (Lasley and Gilbert, 2002).

Simons (1993b) has reviewed the ability of Pb^{2+} to disturb intracellular Ca^{2+} homeostasis, and has emphasized the importance of utilizing free Pb^{2+} concentrations to define Pb^{2+}-Ca^{2+} interactions clearly. Multiple laboratories have investigated the inhibition of depolarization-induced Ca^{2+} currents produced by acute exposure of cultured cells using this approach, resulting in free Pb^{2+} IC_{50} values in the range of 0.3 to 1.3 µM (e.g., Audesirk and Audesirk, 1991; Sun and Suszkiw, 1995). Other workers examined the stimulation of spontaneous transmitter release by acute exposure of permeabilized synaptosomes or cultured cells (Shao and Suszkiw, 1991; Tomsig and Suszkiw, 1996) and reported a free Pb^{2+} EC_{50} of 4 nM. Westerink and Vijverberg (2002) addressed this same question using fluorescent dyes and confocal laser scanning microscopy of permeabilized PC12 cells, an independent approach also based on determination of free Pb^{2+} concentrations. They observed a threshold for acute Pb^{2+} to induce exocytosis of between 10 and 20 nM. Suszkiw (2004) has reviewed this literature and has suggested that Pb^{2+}-induced augmentation of spontaneous release may involve stimulation of vesicle mobilization consequent to Pb^{2+} activation of CaMKII-dependent phosphorylation of synapsin I

and/or stimulation of asynchronous exocytosis via direct Pb^{2+} activation of the putative exocytotic Ca^{2+}-sensor protein synaptotagmin I. Other Ca^{2+}-dependent proteins whose actions are stimulated by Pb^{2+} include calmodulin and calmodulin-dependent phosphodiesterase (reviewed by Goldstein, 1993), calcineurin (Kern and Audesirk, 2000), and Ca^{2+}-ATPase (Ferguson et al., 2000). These actions of Pb^{2+}, shown in Figure 5-3, are proposed to be the points of initiation of much of the metal's cellular toxicity.

Pb^{2+} and Protein Kinase C

As mentioned above, another intriguing focus area for Pb neurotoxicity research has been the interactions of Pb^{2+} with protein kinases. Protein kinase (PKC), a family of serine/threonine protein kinases, has been shown to be targets of Pb^{2+} neurotoxicity. Markovac and Goldstein (1988a) were the first to report that Pb^{2+} directly stimulated PKC activity at picomolar concentrations, thereby exhibiting greater potency for this action than Ca^{2+} by 4 to 5 orders of magnitude. Long et al. (1994) made similar observations using free Pb^{2+} and Ca^{2+} ion concentrations and nuclear magnetic resonance spectroscopy, finding an EC_{50} of 55 pM for Pb^{2+} stimulation of PKC. These workers also presented evidence suggesting that the maximal efficacy of Pb^{2+} was less than that of Ca^{2+}, despite its greater potency. Tomsig and Suszkiw (1995) further elucidated multiple interactions of Pb^{2+} with PKC, identifying both stimulatory (affinity in the picomolar range) and inhibitory (affinities in the nanomolar and micromolar range) binding sites on the kinase. They also showed that on the basis of these interactions, Pb^{2+} induced a peak efficacy for stimulation of PKC that was only ~40% of the maximal efficacy produced by Ca^{2+}, leading them to refer to Pb^{2+} as a partial agonist of the kinase as reflected in Figure 5-4.

The effects of chronic Pb exposure on PKC signaling have been more difficult to evaluate. Most investigators have utilized broken cell preparations and measures of either kinase translocation or enzyme activity; however, the broken cell preparation has not been shown to simulate the intracellular milieu of a chronically exposed intact animal. In the preparation of a tissue extract for determination of kinase activity, the unbound Pb^{2+} is removed or greatly diluted, so that the resulting activity measure largely reflects changes in total PKC expression resulting from the exposure. That is, this measure does not identify a synaptic pool of PKC or necessarily represent the pool of kinase involved in signal transduction. Alternatively, the

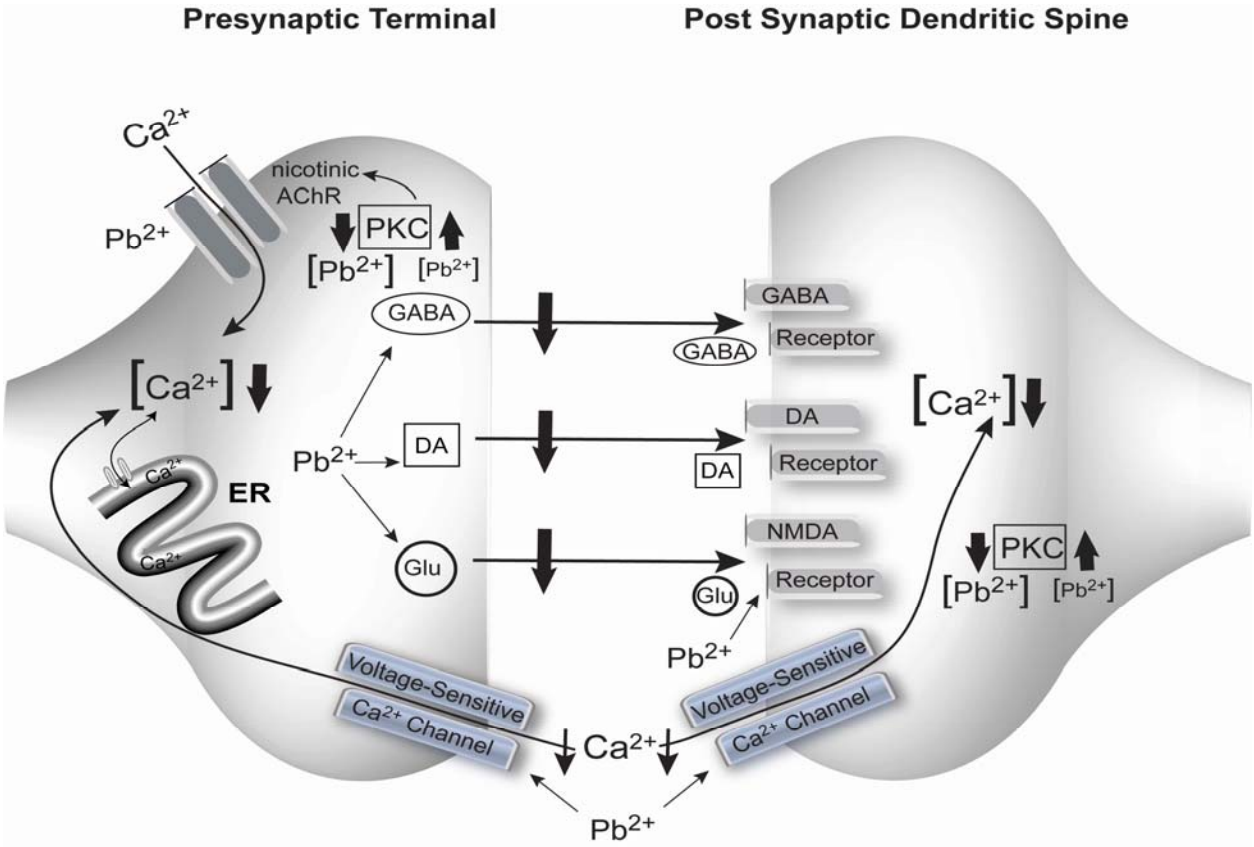

Figure 5-3. Simplified diagram showing the actions of lead at a synapse. Lead decreases release of GABA, dopamine, and glutamate and also decreases Ca^{2+} movement through voltage-sensitive Ca^{2+} channels. Low levels of Pb increase PKC, while higher concentrations inhibit the enzyme. GABA, γ-aminobutyric acid; ER, endoplasmic reticulum; DA, dopamine; Glu, glutamate; PKC, protein kinase C; NMDA, *N*-methyl-D-aspartate.

translocation of kinase from a cytosolic to membrane cellular fraction is a somewhat nonspecific measure, and observed changes should be independently confirmed. From the effects of acute Pb^{2+} exposure in vitro, it seems clear that PKC is a toxicologically significant intracellular target for Pb^{2+}. However, various investigators have been unable to define how this acute effect translates, if at all, to chronic exposure in the intact animal. Neither is it evident how one could discriminate inhibition of PKC activity (due to decreased efficacy relative to that associated with Ca^{2+}, for example) from downregulation of the enzyme due to prolonged stimulation. Resolution of these issues awaits the development of more specific methodologies.

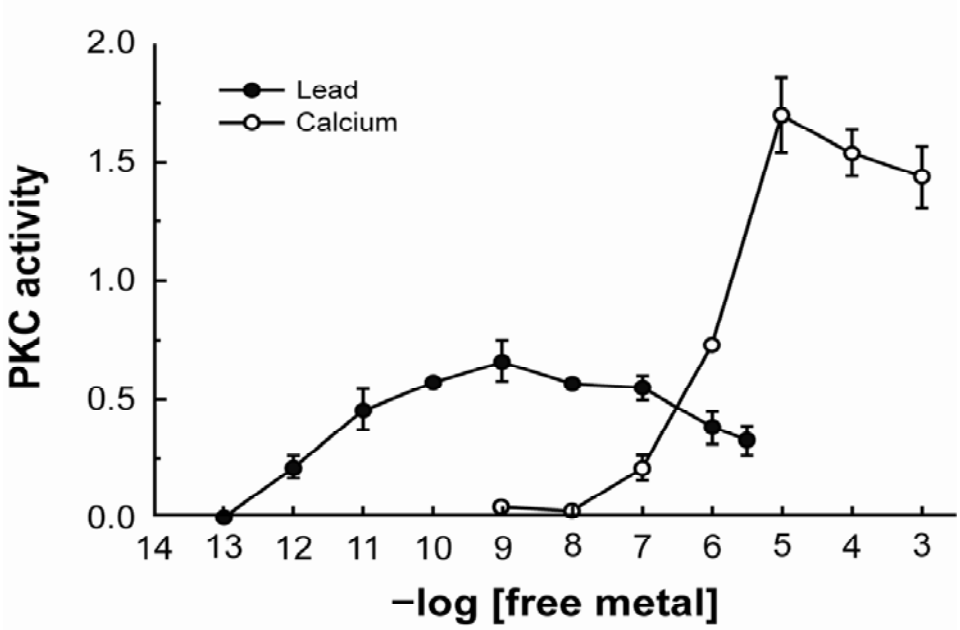

Figure 5-4. PKC activity as a function of Ca^{2+} and Pb^{2+} concentrations.

Source: Tomsig and Suszkiw (1995).

Lead Exposure and Cholinergic Neuronal Systems

The actions of chronic Pb exposure have also been studied with respect to changes in CNS cholinergic systems as another substrate thought to underlie cognitive function. Bielarczyk et al. (1996) reported (1) decreased functional cholinergic innervation in the hippocampus and (2) depression of choline acetyltransferase activity in the hippocampus and cortex of young adult rats exposed to Pb only during early development. This model produced a blood Pb level of 22 µg/dL at the end of exposure. Similar changes were reported by Bourjeily and Suszkiw (1997) in which blood Pb levels were ~20 µg/dL, leading to the conclusion that perinatal Pb exposure results in a loss of septohippocampal cholinergic projection neurons that persists until testing in young adulthood. Tian et al. (2000) exposed PC12 cells to Pb^{2+} for ≤48 h and found that the downregulation of choline acetyltransferase activity reflected the effects of the metal at the level of gene expression. Consistent with these findings, Jett et al. (2002) employed a similar perinatal exposure protocol, producing a blood Pb of 47 µg/dL at the end of exposure. They observed increased nicotinic receptor binding in multiple brain regions. Zhou and Suszkiw (2004) found that acute systemic nicotine reversed a deficit in spatial learning observed in the

offspring of maternally Pb-exposed rats, presumably by compensating for deficient nicotinic function. However, blood Pb levels in the Pb-exposed animals were not reported. These reports reinforce the belief that Pb exposure during early development impacts cholinergic function and suggest that these actions may comprise a component of the cognitive impairment resulting from exposure to the metal.

5.3.3 Actions of Lead Exposure Defined by Neurophysiologic Approaches

An important advance in Pb neurotoxicity research over the past two decades is the widespread application of synaptic plasticity models to study of the effects of exposure. These plasticity models have served as an intermediate link between biochemical and behavioral assessments in that they demonstrate the functional importance of underlying neurochemical mechanisms. Moreover, these models are thought to involve the same physiological substrates as do behavioral paradigms examining cognitive function. Key studies are summarized in Table AX5-3.1.

Chronic Lead and Models of Synaptic Plasticity

About 1990, the LTP model of synaptic plasticity began to be used to study Pb neurotoxicity to evaluate the synaptic processes involved in learning and cognitive function. These investigations have characterized the actions of chronic exposure across several experimental parameters (see Table 5-1). Furthermore, there was uniform agreement as to the alterations that resulted in the hippocampal CA1 and dentate gyrus subregions.

Chronic developmental Pb exposure decreased the magnitude of LTP and increased the threshold for LTP induction (Altmann et al., 1993; Gilbert et al., 1996; Gutowski et al., 1998; Ruan et al., 1998). Simultaneous assessments of paired-pulse functions also uncovered reductions in paired-pulse facilitation, indicating reduced glutamate release (Lasley and Gilbert, 1996; Ruan et al., 1998). It was also shown that the potentiation produced in Pb-exposed animals decayed more rapidly than in controls (Gilbert and Mack, 1998). Blood and brain Pb values reported in these studies are shown in Table 5-1; Lasley and Gilbert (1996) reported the same values as shown for Gilbert et al. (1996).

Gilbert et al. (1999a) compared the effects on LTP when exposure occurred during different developmental periods (see Table 5-1 for blood and brain Pb levels). These workers

Table 5-1. Chronic Lead Exposure and LTP

Recording Site	Exposure Period[1]	Blood Pb[2]	Brain Pb[3]	Preparation	Effect of Exposure on LTP
Hippocampal Dentate Gyrus					
Gilbert et al. (1996)	P0 – P90-120	37.2	ND	In vivo	Elevated induction threshold
Ruan et al. (1998)	P0 – P90-115	30.1	180	In vivo	Diminished magnitude
Gilbert et al. (1999a)	G16 – P130-210 P30 – P130-210	40.2 38.7	378 350	In vivo	Elevated induction threshold and diminished magnitude
Gilbert et al. (1999b)	G16 – P120-180	26.8[4] 40.2 61.8	220 378 670	In vivo	Elevated induction threshold and diminished magnitude
Gilbert and Mack (1998)	G16 – P210-540	ND	ND	In vivo	Accelerated decay
Hippocampal CA1					
Altmann et al. (1993)	G0 – P70-210	14.3	160	slices	Blocked, required exposure during early development
Gutowski et al. (1998)	G0 – P90-130	16.0	135	Slices	Diminished magnitude
Hippocampal CA3					
Gutowski et al. (1997)	G0 – P13-140	28.5	180	Slices	No effect across 4 ages
Gutowski et al. (1998)	G0 – P90-130	16.0	135	Slices	No effect

[1]Exposure duration in terms of gestational (G) or postnatal (P) days of age; P0 = day of birth.
[2]Values expressed as µg/dL.
[3]Values expressed as ng/g tissue.
[4]Different blood Pb values generated by differing levels of exposure.
ND = Not determined

found that animals whose exposure began shortly after weaning exhibited the same impairments in LTP as animals continuously exposed from late gestation when testing in both groups occurred well into adulthood. A smaller effect on potentiation was observed when exposure was restricted to the period from late gestation to weaning. In this study, exposure duration varied significantly, from 26 to >200 days among treatment groups, which suggests the relative importance of duration of exposure in addition to period of exposure for the creation of Pb-induced deficits.

Gilbert et al. (1999b) also examined the effects of Pb on LTP as a function of chronic exposure level, using a range of 0.1 to 1.0% Pb in the drinking water (corresponding to PbB values of 27 to 118 µg/dL; brain Pb measures are shown in Table 5-1). A reduced capacity for LTP was found at all exposure levels except in the 1.0% group, indicating a biphasic dose-effect relationship (Figure 5-5). The 1.0% Pb-exposure level was clearly less effective than the lower exposure groups in reducing LTP magnitude and did not differ significantly from control values. Blood Pb values were elevated as a function of increasing exposure and could not account for the lack of effect in the 1.0% exposure group.

Zhao et al. (1999) utilized low frequency electrical stimulation in the paradigm of long-term depression (LTD) and found that chronic Pb exposure, producing a blood Pb level of 30 µg/dL, depressed the magnitude of this form of synaptic plasticity in both hippocampal CA1 and dentate gyrus subregions. The authors also concluded that in combination with the reduced magnitude of LTP as reported by other workers, the decrease in LTD magnitude results in a reduced range of synaptic plasticity in chronically exposed subjects.

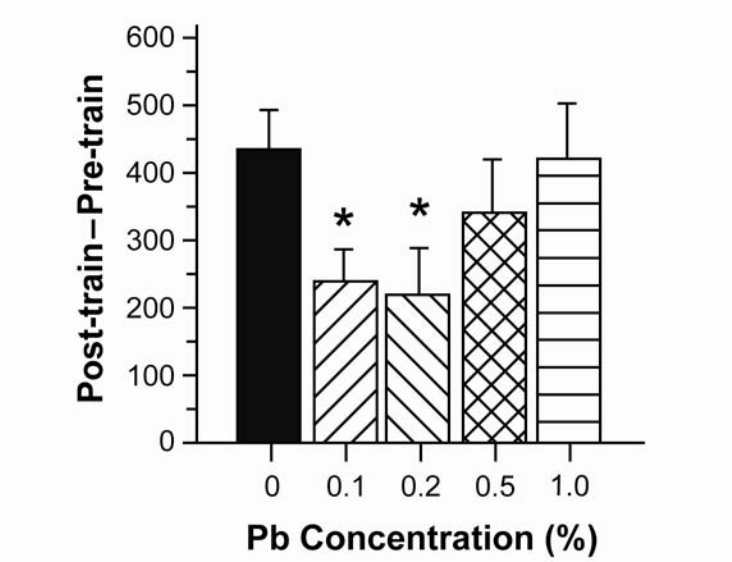

Figure 5-5. Difference score measure of population spike amplitude.

Source: Gilbert et al. (1999b).

While the effects of Pb on synaptic plasticity are quite similar in the CA1 and dentate gyrus, they are not uniformly present throughout this region of the hippocampus. Gutowski et al. (1997, 1998) were unable to find any effect of chronic Pb exposure on LTP in hippocampal CA3 (i.e., mossy fiber LTP), even when the investigation was extended across multiple ages (see Table 5-1 for blood and brain Pb values). The bases for this regional distinction await future investigation.

Lead Exposure, Glutamatergic Transmission, and Synaptic Plasticity

Investigation of the synaptic processes underlying LTP has provided insight into the bases for Pb exposure-induced impairment of potentiation and cognitive ability (reviewed by Lasley and Gilbert, 2000). Biochemical and neurophysiologic approaches (Lasley and Gilbert, 1996; Gilbert et al., 1996; Ruan et al., 1998) have found stimulated glutamate release to be diminished in the hippocampus at PbB values where deficits in LTP have been observed. Multiple actions of Pb may be involved at this exposure level, because animals exposed postweaning exhibited similar decrements in evoked glutamate release to those exposed continuously from conception (Lasley et al., 1999; adult blood Pb values of 39 to 45 µg/dL), similar to the observations for measures of LTP (Gilbert et al., 1999a). A biphasic dose-effect relationship was also found in which stimulated glutamate release in the hippocampus was decreased at intermediate exposures (blood Pb of 27 to 40 µg/dL), but not at higher levels (blood Pb of 62 to 117 µg/dL) (Lasley and Gilbert, 2002). On the basis of these observations, it appears that decreases in stimulated glutamate release may contribute to the biphasic dose-effect relationship in LTP.

In comparison to the high concordance across laboratories with regard to the effects of chronic Pb exposure on LTP and the notable similarities to its actions on glutamate release, there is no general agreement as to the exposure-induced changes in the NMDA receptor. Alterations in receptor function occur readily in response to externally applied treatments and might be expected to vary in a dynamic fashion as a function of exposure parameters, e.g., Lasley et al. (2001) reported receptor upregulation at blood Pb levels in the range of 39 to 62 µg/dL. However, most studies have involved measures of NMDA receptor expression binding in adult animals exposed to constant levels of Pb for at least 3, and more commonly for 6 to 15 months, so that receptor-mediated effects should have stabilized. Consequently, the following alternative conclusions could be proposed regarding the actions of Pb exposure on the NMDA receptor that

are related to its effects on LTP. First, changes in NMDA receptor function may depend on specific Pb exposure conditions. For example, a postweaning exposure protocol may not necessarily produce similar effects to an exposure protocol initiated during earlier development. Alternatively, effects on LTP may be produced at signal transduction or other cellular loci that exert regulatory influences on the NMDA receptor. This latter conclusion implies that changes in the NMDA receptor do not mediate the primary action of Pb on LTP. Furthermore, this suggests that identification of some site of direct Pb effect that has regulatory influence on the receptor would produce more consistently observable findings.

Lead and Electrophysiologic Changes in Dopaminergic/Cholinergic Systems

Electrophysiologic approaches have been employed to delineate other interesting findings in Pb-exposed animals not directly related to synaptic plasticity. Using standard extracellular recording methods, Tavakoli-Nezhad et al. (2001) identified an exposure-dependent decrease in the number of spontaneously active dopamine cells in the substantia nigra and ventral tegmental area in the presence of blood Pb levels of 29 to 54 μg/dL, but they found no evidence that this decrease was related to a physical loss of cells. In subsequent work, Tavakoli-Nezhad and Pitts (2005) determined that the decrease in the number of active dopamine cells in the presence of blood Pb values of 31 μg/dL was not based on depolarization inactivation. However, they discerned a reduced impulse flow in dopamine neurons and a diminished sensitivity of D_1 receptors in the nucleus accumbens. The functional importance of these observations remains to be determined.

The actions of Pb^{2+} on cholinergic nicotinic receptors have been investigated in acutely dissociated or cultured hippocampal cells using the patch clamp technique in whole cell mode (Ishihara et al., 1995). These workers found that Pb^{2+} potently inhibits activation of fast-desensitizing nicotinic currents in a noncompetitive and voltage-dependent manner. The nicotinic receptors affected (methyllycaconitine-sensitive) were more sensitive to Pb^{2+} than other nicotinic subtypes and are known to be highly permeable to Ca^{2+}. This latter observation likely explains the potency for their inhibition by Pb^{2+}.

5.3.4 Lead Exposure and Sensory Organ Function

Research assessed in the 1986 AQCD demonstrated that the visual system is sensitive to perturbation by neonatal Pb exposure. Suckling rats exposed through dams' milk creating blood Pb values of 65 µg/dL at postnatal day (PND) 21 had significant alterations in their visual-evoked responses and decreased visual acuity, indicating depressed conduction velocities in visual pathways. It was hypothesized that neonatal lead exposure increases the ratio of excitatory to inhibitory systems in the developing cerebrospinal axis and decreases the number of cholinergic receptors, leading to lasting decreases in visual acuity and spatial resolution.

Sensory organ function has continued to be a productive focus area for Pb neurotoxicity research, generating important scientific findings. Visual and auditory systems have received the most attention, have generated results closely resembling clinical observations, and have been successful in defining some of the mechanisms underlying the exposure-induced alterations. These studies are summarized in Table AX5-3.3.

Sensory Organ Assessments in Nonhuman Primates

Lilienthal and Winneke (1996) tested monkeys continuously exposed to Pb from gestation through 8 to 9 years of age, producing blood Pb values of 33 to 56 µg/dL before termination of exposure. They found increased latencies for waves I, II, and IV in brainstem auditory evoked potentials. These effects persisted for at least 18 months after exposure was terminated and blood Pb values had declined nearly to control levels. Rice (1997) determined pure tone detection thresholds in monkeys exposed continually from birth to 13 years of age, resulting in blood Pb levels of 30 µg/dL from 3 to 9 years of age and 50 to 170 µg/dL around the time of testing. Half of the subjects exhibited thresholds outside of the control range at some frequencies. These findings are consistent with reported alterations in auditory function in humans developmentally exposed to Pb (reviewed by Otto and Fox, 1993). Moreover, these authors concluded that evidence from human and animal studies indicate that Pb exposure impairs auditory function. In both developing and mature humans and experimental animals, the cochlear nerve and more central structures appear to be preferentially sensitive. At low to moderate levels of Pb exposure, consistent findings include elevations in hearing thresholds and increased latencies in brainstem auditory evoked potentials. Thus, there is good correspondence between human and animal studies in the effects of chronic Pb on auditory function.

Visual pathology was assessed by Reuhl et al. (1989) in monkeys by exposing low- and high-dose groups from birth to 6 years of age. Blood Pb values were 10 and 50 µg/dL, respectively, except for a 3- to 4-month period, when values rose to 20 and 220 µg/dL. This investigation uncovered a decrease in neuronal volume density in cortical areas V1 and V2 in the high-exposure compared to the low-exposure group, and also a decrease in dendritic arborization in pyramidal neurons in these brain areas. These authors concluded that chronic developmental Pb exposure produces changes in cytoarchitecture in visual projection areas. Lilienthal et al. (1988) continuously exposed monkeys to 350- or 600-ppm Pb acetate beginning prenatally, producing blood Pb values of ~40 and 50 µg/dL, respectively. Visual evoked potentials and electroretinograms (ERG) were recorded at ~7 years of age. Exposure-related decreases in amplitudes and increases in latencies were observed. In Pb-exposed monkeys, the effects on amplitude were greater in dark conditions, and the effects on latencies were greater in bright conditions. Electroretinograms, studied during dark adaptation, showed greater increases in amplitude of the b-wave in exposed animals. Thus, visual function in primates is also impaired as a result of exposure.

Retinal Function in Rodents

The actions of Pb on retinal cells have been a focus of research for more than two decades. It has long been recognized that Pb^{2+} exhibits a selective effect on rod cells (Fox and Sillman, 1979) and, more recently, that the associated loss of rod and bipolar cells was due to exposure-induced apoptotic changes (e.g., Fox et al., 1997, in the presence of blood Pb values of 19 to 59 µg/dL at the termination of exposure). These observations have been linked with exposure-related alterations in rod-mediated visual function. In vitro studies using free Pb^{2+} ion concentrations have done much to elucidate the mechanistic bases of these observations.

These latter efforts have established the concentration-dependent inhibition of cyclic GMP (cGMP) hydrolysis by free Pb^{2+}, in addition to increases in retinal cGMP and rod Ca^{2+} levels (e.g., Srivastava et al., 1995). Kinetic studies have shown that picomolar Pb^{2+} concentrations competitively inhibit rod cGMP phosphodiesterase relative to the millimolar concentrations that are required for Mg^{2+} cofactor activity, thus binding with 10^4- to 10^6-fold higher affinity than Mg^{2+} and preventing cGMP hydrolysis (Srivastava et al., 1995). When retinas are incubated in Ca^{2+} and/or Pb^{2+} in vitro, the rods selectively die by apoptosis associated

with mitochondrial depolarization, release of mitochondrial cytochrome *c*, and increased caspase activity (He et al., 2000). He et al. (2003) have proposed that apoptosis is triggered by Ca^{2+} and Pb^{2+} overload resulting from translocation of cytosolic Bax to the mitochondria, which likely sensitized the overloaded mitochondria to release cytochrome *c*. This effect occurred at a blood Pb level of 26 µg/dL at the end of exposure. Subsequent work found the elevations in free Ca^{2+} and Pb^{2+} to be localized to photoreceptors and determined that the effects of the two ions were additive and blocked by a mitochondrial permeability transition pore inhibitor (He et al., 2000). This suggested that the two ions bind to the internal metal binding site of this pore and, thereby, initiate the apoptosis cascade.

These mechanisms are consistent with ERG changes observed in animals chronically exposed during early development: decreases in maximal ERG amplitude, decreases in absolute ERG sensitivity, and increases in mean ERG latency that were selective for rod photoreceptors in the presence of blood Pb values of 59 µg/dL at the termination of exposure (Fox and Farber, 1988). Also in agreement with these mechanisms were observed elevations in retinal cGMP levels and reductions in light-activated cGMP phosphodiesterase activity. Moreover, the degenerating rod and bipolar cells exhibited the classical morphological features of apoptotic cell death (Fox et al., 1997). Other measures of visual function in chronically exposed animals also have been found to be consistent with the mechanistic data. Long-term dose-dependent elevations in response thresholds were observed only at scotopic (i.e., rod-mediated) levels of illumination, and dark adaptation was delayed (Fox et al., 1994; in the presence of blood Pb values of 19 to 59 µg/dL at the termination of exposure). In addition, exposure-induced decreases in rhodopsin content that were proportional to the loss of rod cells have been reported (Fox et al., 1997) as well as dose-dependent decreases in retinal Na, K- ATPase activity (Fox et al., 1991a; blood Pb levels as above in Fox et al., 1994).

The studies investigating rod photoreceptors are perhaps the best examples of the ability to correlate data obtained in vitro with findings derived from in vivo exposure and with changes in visual physiology. In multiple instances, the same cellular mechanisms were affected with each approach and are consistent with ERG and rod-mediated functional measures. These relationships are summarized in Table 5-2.

Table 5-2. Mechanisms of Lead-Induced Impairment of Retinal Function

In Vitro Evidence	In Vivo Evidence	Physiologic Changes
Competitive inhibition of cGMP PDE	Decreased stimulated cGMP PDE activity	
Increased retinal cGMP	Increased retinal cGMP	Decreased maximal ERG amplitude Decreased absolute ERG sensitivity Increased mean ERG latency
Increased rod [Ca^{2+}]		
Apoptosis from increased photoreceptor Ca^{2+}/Pb^{2+} via binding to mitochondrial permeability transition pore	Morphological features of apoptotic rod, bipolar cell death Decreased rhodopsin proportional to cell loss Translocated cytosolic Bax to the mitochondria, cytochrome *c* released	Increased response thresholds at scotopic backgrounds Delayed dark adaptation
Decreased retinal Na^+, K^+-ATPase activity	Decreased retinal Na^+,K^+-ATPase activity	

Abbreviations: PDE, phosphodiesterase; ERG, electroretinogram.

5.3.5 Neurobehavioral Toxicity Resulting from Lead Exposure

As discussed elsewhere in this chapter, the young are vulnerable to the effects of Pb exposure due to their greater absorption and retention of Pb. The developing state of the nervous system makes the perinatal period a particularly critical time for the initiation of neurobehavioral perturbations by exposure to Pb. However, work reviewed in the 1986 Lead AQCD showed that behavioral effects in animals are found with both perinatal exposures and with exposures after weaning or during adulthood.

Very early research on the effects of Pb on learning ability failed to adequately report exposure regimen or the resulting body burden. Studies reviewed in the 1986 AQCD were more useful; they reported this information and further attempted to control for the confounding factors of litter size and undernutrition. Thirty-four rat studies were evaluated, from which it was possible to ascertain that learning was altered at blood Pb levels of 15 to 30 µg/dL. Test methods that revealed learning deficits in rats included radial arm maze testing and fixed-interval (FI) operant conditioning. Rats in these studies tended to respond more rapidly (i.e., higher

response rates, shorter interresponse times, or shorter response latencies) or to respond even when inappropriate (i.e., when no reward is provided for responses or when reward is specifically withheld for responding). Impaired acquisition of discrimination and performance in other tests has been demonstrated with similar blood Pb levels in rats.

Thirteen nonhuman primate studies previously reviewed showed that exposures from birth impaired learning ability, even after current blood Pb levels had dropped to control values. Studies using operant conditioning tasks demonstrated that learning ability was impaired when monkeys' blood Pb levels reached 15 µg/dL and steady state levels were 11 µg/dL. Other significant findings in these studies, consistent with the rat findings mentioned above, were the tendency for Pb-induced excessive or inappropriate responses in the monkeys and higher response rates and shorter interresponse times on FI operant schedules. The neural mechanisms responsible for this "hyperreactive" behavior were thought to originate in the hippocampus, as similar behaviors have been shown in animals with lesions of that brain region. These increased response tendencies were shown to change to decreased responding with sufficiently high exposure levels. An explanation of this curvilinear dose-response was that there are differences in the time required for response rates to reach their maximum as a function of different exposure levels. At sufficiently toxic Pb concentrations, responding declines due to the inability to perform the necessary motor responses.

A survey of the major animal studies published since the 1986 AQCD that characterized Pb-induced neurobehavioral deficits that may correlate with behavioral deficits observed in humans are presented below, organized by endpoint, test method, and species. Summaries of key animal neurobehavioral studies are presented in Annex Table AX5-3.4.

Effects of Pb on Learning Ability

In the past 20 years, major advances have occurred in the understanding of the effects of Pb on learning ability, which is impacted throughout the life cycle. Assessments of Pb-induced deficits in learning ability in both rats and primates are discussed below.

Schedule-Controlled Behavior

Schedule-controlled behavior studies, such as fixed interval (FI) and fixed ratio (FR) operant conditioning, have been used with both rats and monkeys to assess cognitive ability

(integrated with sensory and motor abilities). The effects of prolonged Pb exposure on FR performance was evaluated in Long Evans (LE) rats exposed throughout the study to 50 or 500 ppm from weaning, producing blood Pb levels of 30.3 and 58 to 94 µg/dL, respectively (Cory-Slechta, 1986). At PND 55, assessment of FR performance was started using increasing ratio values. No effects were seen in the 50-ppm group. In the 500-ppm group, response rates initially decreased, then reached control levels, primarily because of longer interresponse times (IRTs). In comparing these data to earlier studies with similar Pb exposures, the author concluded that FI response rates are more sensitive to perturbation by Pb than FR response rates.

To evaluate the effects of exposure duration on FI performance, PND 21 LE rats were exposed to 50 ppm Pb for 8 to 11 months, then tested using an FI 1-min schedule of food reinforcement (Cory-Slechta, 1990a). These rats demonstrated decreased FI response rates (i.e., longer IRTs and lower running rates) compared to controls. The author suggested that this suppression of FI response rates, which contrasted with earlier studies showing increased response rates with shorter exposures but similar blood Pb levels (~20 µg/dL), was due to the greater body and brain burdens of Pb. Following changes in schedule parameters, Pb-treated rats demonstrated a delay in acquisition. In the same study, adult rats (6 to 8.5 months at exposure) were trained on FI schedules and then exposed to 50 or 500 ppm Pb for 3 to 5 months. These animals demonstrated no consistent changes in FI performance, suggesting that once a behavior has been acquired, it may be resistant to disruption by subsequent Pb exposure.

To examine old age as a possible vulnerable period for Pb exposure, Cory-Slechta and Pokora (1991) dosed Fischer 344 (F344) rats at PND 21, 8-months of age (adult), and 16-months of age (old) with 2 or 10 mL/kg/day Pb acetate for 9.5 months. Training began 2.5 months after the start of exposure. Steady state blood Pb levels of 13 to 18 µg/dL were obtained. Young and old rats demonstrated increased variable-interval (VI) and FI response rates, while adult rats showed decreased response rates on both schedules. Effects on FI responding were seen with the 2 mg dose and on VI with only the 10 mg dose. Additionally, these data suggest that F344 rats are less sensitive to Pb effects than the LE rats used in most of the previous schedule-controlled behavior studies.

To characterize neurotransmitter system involvement in Pb-induced changes in FI performance, rats were exposed from weaning to 0, 50, or 150 ppm Pb acetate, resulting in blood Pb levels of ~<5, 15 to 25, and 30 to 50 µg/dL, respectively (Cory-Slechta et al., 1996b).

Behavior was shaped at PND 40 to 45 days, followed by imposition a FI 2-min schedule of reinforcement. Dopaminergic (DA) agonists quinpirole (D_2), SKF38393 (D_1), and SKF82958 (D_1); μ-opioid agonist morphine; muscarinic cholinergic agonist arecoline; glutamate agonist NMDA; and NMDA antagonist MK801 were administered after 50 FI sessions. FI performance was altered by all drugs tested except NMDA. Pb exposures attenuated the decrements in rates produced by the two D_1 agonists and, at 150 ppm Pb exposure, altered the rate change associated with the low dose (0.03 mg/kg) of quinpirole. The DA agonists' effects were not concentration-dependent. These data suggest that Pb-induced changes in behavior were mediated by D_1 receptors. Additional evidence suggesting Pb's attenuation of DA activity was obtained using the D_2 agonist quinpirole and the D_2 antagonist eticlopride (Areola and Jadhav, 2001). Post-weaning, rats that had been exposed to 50-ppm lead acetate, producing a blood Pb level of 15.1 μg/dL, were tested on an FI 1-min schedule. Quinpirole at 0.05 mg/kg reversed the effects of Pb, while eticlopride (0.01 and 0.05 mg/kg) had no effect on response rates in Pb-treated animals.

To test the hypothesis that elevated nucleus accumbens (NAC) DA is a mechanism of Pb-induced changes in FI performance, NAC DA activity was evaluated using the DA antagonist N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinone (EEDQ) (Cory-Slechta et al., 1998). LE rats were exposed to 0, 50, or 500 ppm Pb acetate continuously from weaning, creating blood Pb levels of 2.1/0.5, 7.2/9.6, and 49.1/49.4 μg/dL (~3 months of exposure/end of experiment). After shaping (lever press), rats performed an FI 1-min schedule of reinforcement for at least 50 sessions. DA increased FI rates in the 0 and 50 ppm groups and decreased rates in the 500-ppm group. Intra-NAC administration of EEDQ suppressed FI response rates. At the highest EEDQ dose, Pb at 500 ppm delayed recovery of response rates to control level rates, suggesting that NAC DA activity may be one mechanism mediating FI response rates. Using a similar exposure paradigm, Cory Slechta et al. (2002a) examined the involvement of the dorsomedial striatum in Pb-induced increases in FI response rates. Both DA and EEDQ, microinjected into the dorsomedial striatum, increased or decreased FI response rates, which depended on baseline FI overall rates. DA mimicked the effects of Pb in this region. At this point, it is unclear whether this area of the striatum modulates Pb-induced changes in FI performance. Changes in FI performance were also used to characterize interactions between chronic Pb exposure and intermittent stress (Virgolini et al., 2005), discussed below.

Rice (1988a) orally dosed cynomolgus monkeys from birth with 2 mg/kg/day of Pb acetate continuously throughout the study. At PND 100, blood Pb levels peaked at 115 µg/dL and declined to 33 µg/dL by PND 270. At PND 60, the monkeys were tested on an FR schedule, learning to respond by pushing a button to receive a reinforcement. The Pb-treated monkeys at 2.5 to 5.0 months of age demonstrated increased mean FR pause times compared to controls. In later, but not earlier sessions, FI pause was decreased in Pb-treated monkeys. Following FR training, the monkeys were tested on a discrimination reversal, then a chain FR 1min-FI 2 min operant schedule, which required the monkeys to complete the FR, followed by the FI to receive one reinforcer (chain FI-FR). The monkeys were then tested as juveniles (3 years of age) on a multiple FI-FR schedule of reinforcement. Pb-treated juvenile monkeys demonstrated increased FI run rate, pause time, and index of curvature. At both ages, the treated monkeys showed increased variability of performance (both within and between sessions, and between subjects) compared to controls.

To evaluate the effect of Pb exposure during different developmental periods, Rice (1992a) exposed cynomolgus monkeys to 1.5 mg/kg/day Pb acetate either continuously from birth, from birth to PND 400, or from PND 300 onward. These exposures resulted in a steady state blood Pb level of 20 to 35 µg/dL during dosing. Tested at 3 years of age on a multi FI-FR, the Pb-treated monkeys showed no effects on FI rate. Tested at 7 to 8 years of age, all three groups of treated monkeys demonstrated increased run rates and decreased interresponse times on the FI. To explain the negative results in the juveniles and the positive results in the adults, the author postulated a possible interaction of Pb with the behavioral history. The monkeys had been tested first with a multi FI-FR, then a differential reinforcement of low rate (DRL) schedule, a series of nonspatial discrimination reversal tasks, a delayed spatial alternation (DSA) task, and then a second multi FI-FR at 7 to 8 years of age. Additionally, the author stated that FI performance can be affected even without exposure to Pb during infancy and that exposure only during infancy is sufficient to affect responses.

A concurrent schedule of reinforcement was used to test squirrel monkeys exposed gestationally to Pb (Newland et al., 1994). Maternal blood Pb levels ranged from 21 to 79 µg/dL. At 5 to 6 years of age, the monkeys were tested using a concurrent reinforcement with VI schedules. The monkeys were allowed to respond on either of two levers, one of which had a greater density of reinforcement than the other. The ratio of reinforcement density was changed

within the test session trial. Control monkeys learned to follow the reinforcement density by responding to a greater degree to the lever associated with the richer density. Monkeys whose blood Pb level in utero had been ≥40 μg/dL changed their responses more slowly or in the wrong direction to the changing reinforcement, suggesting to the authors that this faulty response to changes in reinforcement may be one mechanism of learning impairment.

Schedule-controlled behavior in squirrel monkeys was assessed at ages 3 to 7 years following in utero-only exposure to Pb acetate (Newland et al., 1996). Doses were adjusted individually to provide a maternal blood Pb level of 21 to 70 μg/dL. The monkeys were trained to pull a 1-kg weighted bar on FR and FI schedules of reinforcement. Pb-treated monkeys demonstrated an increase in the number of responses that failed to adequately displace the bar. This increase in incomplete responses occurred on FR schedules, but not on the FI schedule. The enhanced sensitivity of the FR schedule may be attributed to it requiring a fixed number of responses for reinforcement, unlike the FI schedule, which reinforces only one response for reinforcement. Because the monkeys had to use greater physical force to complete the response than the monkeys in the studies discussed above, this study identified a deficit in the physical execution of the response. The lack of increased response rate could also be related to the physical effort required. These data suggested to the authors that gestational exposure to Pb can produce motor impairments long after exposure has ended and that these motor impairments accompany deficits in acquisition behavior.

In both rats and monkeys, an increased rate of FI responding has been seen with Pb exposures producing blood Pb levels as low as 11 μg/dL. Figure 5-6 shows a graph summarizing studies examining Pb-induced changes in FI response rates (Cory-Slechta, 1994). This figure summarizes the dose effect function for Pb-induced changes in FI performance for several species. Low-level Pb exposures increase FI response rates and high-level Pb exposures decrease FI response rates. These data extend earlier findings of a curvilinear dose-response relationship for this endpoint.

Differential Reinforcement of Low Rates

On the basis of the results of the FI testing done on monkeys described above, Rice (1992b) used a DRL to assess whether the monkeys could learn to inhibit inappropriate responding. Monkeys were exposed to 2 mg/kg/day Pb acetate, creating a steady state blood Pb

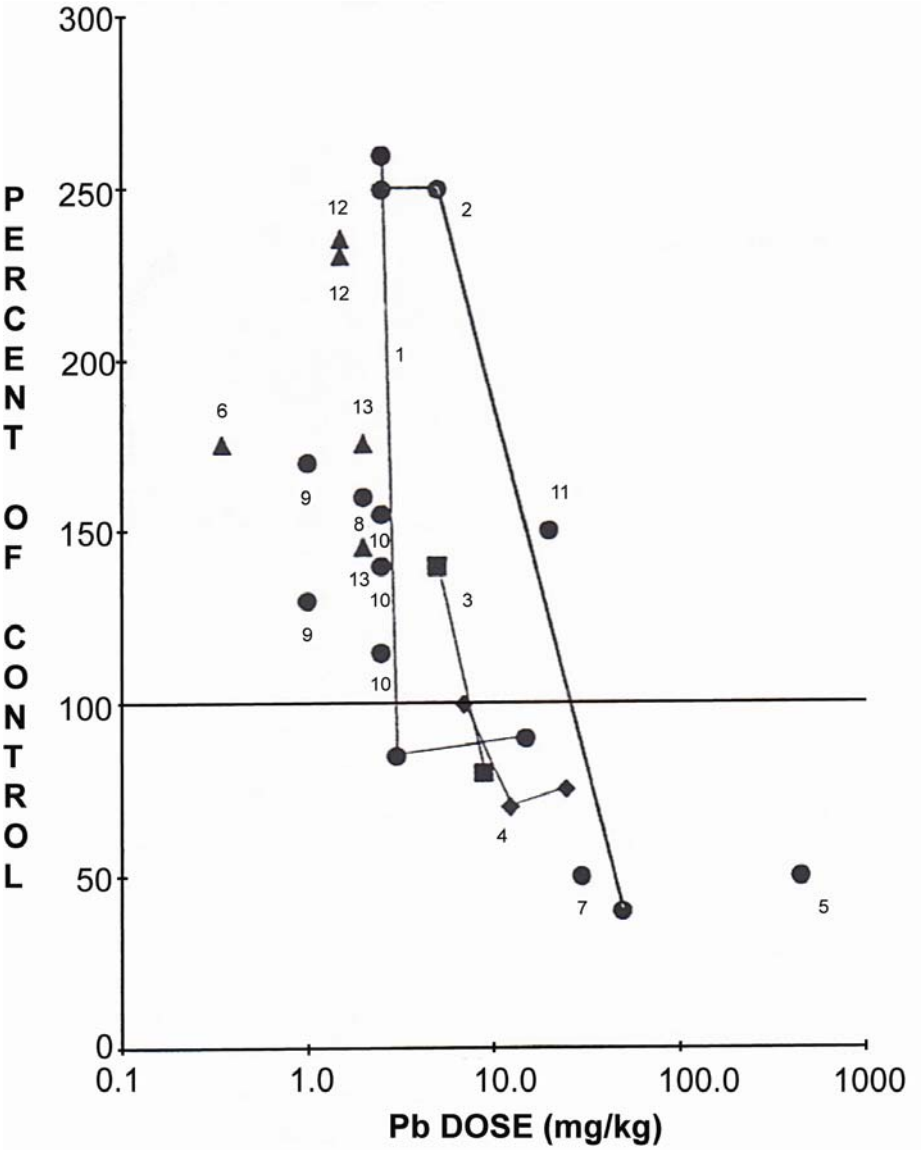

Figure 5-6. Dose-effect function for lead-induced changes in fixed-interval performance. The lead effect (response rate, interresponse time, or percentage of reinforcement) was plotted as a percentage of the control group value for sessions in which peak effects were observed. Different symbols represent different experimental species: circles, rats; triangles, monkeys; squares, sheep; diamonds, pigeons. Numbers next to curves or selected points represent data from the following studies: (1) Cory-Slechta et al., 1983; (2) Cory-Slechta and Thompson, 1979; (3) Van Gelder et al., 1973; (4) Barthalmus et al., 1977;(5) Zenick et al., 1979; (6) Rice et al., 1979; (7) Angell and Weiss, 1982; (8) Cory-Slechta and Pokora, 1991; (9) Cory-Slechta et al., 1985; (10), Cory-Slechta and Weiss, 1989; (11) Nation et al, 1989; (12) Rice 1992a; (13) Rice, 1988b.

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level of 33 μg/dL after withdrawal of infant formula. Compared to controls, the Pb-treated monkeys demonstrated greater non-reinforced responding, less reinforced responding, and had a shorter average time between responses. These results suggested to the author that Pb exposure may cause a failure to inhibit inappropriate responding, a poorer ability to use internal cues for timing, or an alteration in timing capabilities.

Radial Arm Maze and Passive Avoidance

The radial arm maze evaluates spatial acquisition and retention by measuring animals' retrieval of food from the arms of the maze. A study was done to determine if Pb-induced behavioral deficits in this learning endpoint are related to injury of hippocampal neurons (Munoz et al., 1988). Female Wistar rats were fed 750-ppm Pb acetate in their diet and then bred after 50 days. Pups were either continued on the same Pb diet for permanent exposure or fed control chow for maternal-only exposure. Blood Pb values at PND 16 were 17.3 μg/dL in Pb-exposed rats and ranged from 32 to 39 μg/dL in continuously dosed animals. Brain Pb levels were 7.3 μg/g at PND 16 in Pb-exposed animals. Hippocampal lesions, consisting of complete bilateral depletion of granular and pyramidal cells in dorsal hippocampus, were induced in other rats by stereotaxic injection of ibotenic acid. The lesioned animals showed no effects on acquisition of learning in the radial arm maze, while the Pb-exposed animals did. Tested 4 weeks later, both lesioned and Pb-treated animals showed impaired retention, suggesting to the authors that Pb may damage the dorsal region of the hippocampus and may be associated with the retention component of learning. A subsequent study by the same group (Munoz et al., 1989), using a similar Pb exposure protocol and ibotenic acid lesions to the amygdala, was done to determine if that brain region was involved in Pb-induced learning deficits. Both treatments impaired both acquisition of food-retrieval behavior in the maze and passive avoidance behavior, but neither treatment affected locomotor activity. The permanently exposed rats showed greater deficits, indicating possible reversibility of Pb-induced effects in prenatal-only exposures or the cumulative effects of the chronic exposure creating a greater body burden of Pb. Further, these data point to the amygdala as another Pb target.

Discrimination

Early studies demonstrated Pb-induced impairments in tests of discrimination of relevant environmental stimuli. To study the effects of chronic Pb exposure on discrimination, Morgan et al. (2000) exposed LE rats continuously from the beginning of gestation. Lead acetate at 0, 75, or 300 ppm in drinking water produced adult blood Pb levels of <5, 20, and 36 µg/dL. At ages 7 to 9 weeks, an automated, three-choice visual discrimination task revealed a dose-dependent slowing of learning and an increased incidence of "impaired" animals. The authors concluded that chronic developmental Pb exposure results in associative deficits and an increased tendency to respond rapidly. In another study, Morgan et al. (2001) evaluated Pb-induced alterations in visual discrimination in LE rats exposed only during early development. One group received Pb acetate in drinking water throughout gestation and lactation (GL300), other groups received 300 or 600 ppm Pb during lactation only (L300 and L600). Blood Pb levels were <5 (controls), 36–43 at PND 8, 27–34 at PND24, 131–158 at PND 53, and 16-18 µg/dL at PND 53 (treated animals). Pb-treated animals showed no differences in learning rate, motivation, or response latency for correct or incorrect responses.

Discrimination Reversal

Discrimination reversal studies examine the ability to alter behavior in response to a change in reinforcement contingencies. Earlier studies showed that chronic low-level exposures in monkeys, creating a steady state blood Pb level of 11 to 15 µg/dL, produced deficits in nonspatial discrimination reversal tests, with and without irrelevant cues. Reversal of previous learning appears to be consistently affected by Pb exposure, often resulting in perseverative behavior. Additionally, in early cynomolgus monkey studies, the Pb-treated monkeys were found to be more distracted by irrelevant cues than control monkeys.

Using this same cohort of cynomolgus monkeys, Gilbert and Rice (1987) examined spatial discrimination reversal at 9 to 10 years of age. The monkeys had been exposed to 50 or 100 µg/kg/day Pb acetate, resulting in blood Pb peaks of 15.4 and 25.4 µg/dL, respectively. Steady state blood Pb levels were 10.9 and 13.1 µg/dL, respectively. Compared to controls, the treated monkeys were impaired in the presence, but not the absence, of irrelevant cues. In the lower-dose group monkeys (blood Pb 10.9 µg/dL), impairment ended when the irrelevant stimuli became familiar.

To evaluate the effects of timing of exposure on this learning task, monkeys were exposed to one of three protocols: (1) 1.5 mg/kg/day Pb acetate continuously from birth; (2) during infancy only (birth to PND 400); or (3) beginning after infancy (Pb from PND 300 and thereafter) (Rice and Gilbert, 1990a). These exposures resulted in blood Pb levels of 32 to 36 µg/dL during dosing and given infant formula and levels of 19 to 26 µg/dL during Pb exposure in the post-infancy group. At age 5 to 6 years, the monkeys were tested on a nonspatial discrimination reversal task. Monkeys exposed continuously and those exposed beginning after infancy demonstrated a dose-dependent impairment of learning. The monkeys exposed only during infancy showed no impaired learning of the task. At 7 to 8 years of age (adults), the monkeys were tested on spatial discrimination tasks (Rice, 1990). Using no irrelevant cues or irrelevant form and color cues, results showed that the continuously exposed monkeys were impaired in the absence of irrelevant cues. All three treatment groups were impaired when irrelevant cues were present. These results are in contrast to the results on the nonspatial tasks described by Rice and Gilbert (1990a) and suggested to the author that the developmental period of exposure may differentially affect spatial and nonspatial tasks.

The effects of Pb on olfactory reversal discrimination have been examined in two rodent studies. Hilson and Strupp (1997) exposed LE rats chronically from conception to 0, 75, or 300 ppm Pb acetate in water. Blood Pb levels were, respectively, <5, 26, and 51 µg/dL on PND 1; <5, 22, and 37.5 µg/dL on PND 17; and <5, 27.5, and 51 µg/dL in adulthood. At 20, 14, and 22 weeks of age for the three replicates, testing consisted of acquisition of the original discrimination (i.e., learning to respond to an odor), then reversal learning in which the other odor became correct. Pb treatment did not affect learning the original discrimination; however, it did impair learning the reversals in the high dose group by prolonging the postperseverative phase, which the authors note is similar to the effects seen with lesions of the amygdala. Both groups of Pb-treated rats also showed impairment during an extradimensional shift in which the rats had to learn the correct spatial location of the odor for reward. Garavan et al. (2000) further investigated these responses in LE rats exposed to: (1) 0 ppm Pb; (2) 300 ppm Pb acetate during both gestation and lactation (GL300); (3) 300 ppm during lactation (L300); or (4) 600 ppm during lactation to (L600). At testing on PND 53, blood Pb levels were <5, 16, 12, and 18 µg/dL, respectively. Compared to controls in a two-choice olfactory serial reversal task, all Pb-treated groups needed more trials to reach the point at which perseverative responding to the

previously correct cue ended. The authors hypothesized that this deficit was not due to perseverative responding, but rather to a Pb-related spatial response bias and a concurrent, but independent, associative impairment.

Learning Set Formation

Learning set formation tasks evaluate an animal's ability to "learn to learn" discriminations by presenting a series of visual discrimination problems and quantifying the rate at which each successive problem is learned. To ascertain Pb's effects on this learning endpoint, Lilienthal et al. (1986) exposed rhesus monkeys to either 0 (control), 350 (low,) or 600 ppm (high) Pb in utero, resulting in blood Pb levels of 50 and 110 µg/dL, respectively. At age 12 to 15 months, only the high-dose group exhibited deficits in simple discrimination learning, which the authors attributed to possible Pb-induced reduced attention, higher frustration levels, and lowered adaptability. Both groups showed impairments in forming a learning set, which the authors hypothesize was due to Pb-induced cognitive deficits.

Concurrent Discrimination

Concurrent discrimination tests an animal's ability to learn a second set of problems after a first set was learned concurrently to criterion. Rice (1992c) further evaluated the monkeys used in the discrimination reversal studies described by Rice and Gilbert (1990a). At 8 to 9 years old, the monkeys were tested on two sets of concurrent discrimination tasks. Pb-treated monkeys in all three exposure groups learned more slowly, although there was less impairment in the monkeys exposed only during infancy. Perseverative behavior was also demonstrated in the treated monkeys. These data are consistent with the other discrimination studies.

Repeated Acquisition and Performance Schedule

Another test method effectively utilized to distinguish changes in chronically Pb-exposed animals is the repeated acquisition and performance schedule (Cohn et al., 1993). The purpose of this test is to determine the selectivity of Pb-induced changes in learning, as distinct from nonspecific or performance effects, and to explore the nature of the underlying error patterns contributing to any learning deficits. This schedule required completion of a sequence of three responses for reinforcement, with the correct sequence for the learning component changing with

each successive experimental session (i.e., repeated acquisition). In contrast, the correct sequence remained constant across sessions in the performance component; thus, once learned, this component did not require further learning to complete successfully.

This schedule was used in animals chronically exposed from weaning to Pb acetate at 0, 50, or 250 ppm in drinking water, producing blood Pb levels of 2.8, 25.1, and 73.5 μg/dL, respectively. Pb treatment caused significant decrements in accuracy on the learning component, but not on the performance component, compared to controls (Cohn et al., 1993). A detailed analysis of the subjects' behavior indicated that Pb exposure impaired learning by increasing perseverative responding on a single lever, even though such repetitive responding was not directly reinforced. In a subsequent study using the same exposures, dose-effect curves for the NMDA receptor antagonist MK-801 were determined in controls and animals in which chronic Pb exposure began at weaning (Cohn and Cory-Slechta, 1993). The decline in learning accuracy and the increases in perseverative responding produced by MK-801 were attenuated by Pb exposure, and dose-effect curves relating MK-801 dose to changes in rates of responding were shifted to the right in Pb-exposed rats compared to control animals. These observations demonstrate a subsensitivity of Pb-exposed animals to both the accuracy-impairing and response rate-altering properties of the antagonist. An additional investigation used the same Pb exposure protocol and administration of doses of NMDA as a receptor agonist to rats performing this test (Cohn and Cory-Slechta, 1994a). In control animals, NMDA was found to decrease accuracy of response in both the repeated acquisition and performance components of this multiple schedule and to suppress response rates as well. Pb exposure potentiated the accuracy-impairing effects of NMDA by further increasing the frequencies of errors and likewise potentiated the drug's rate-suppressing effects. Thus, as stated earlier in this section, the Pb-induced potentiation of the agonist effects and reduced sensitivity to the antagonist effects in this test are consistent with a functional upregulation of NMDA receptors in Pb-exposed brain. In other work, Cohn and Cory-Slechta (1994b) were unable to distinguish any evidence of dopaminergic modulation of responding in this behavioral paradigm. Thus, the repeated acquisition and performance schedule proved valuable not only in providing a finer dissection of the animal's behavior, but also in elucidating important mechanistic aspects of Pb neurotoxicity. It also provides an unambiguous indication of an adverse effect (learning impairment) in the absence of sensory and motor deficits.

Avoidance Learning

Altmann et al. (1993) examined the effects of Pb on active avoidance learning (AAL) in the offspring of Wistar rats fed 750-ppm Pb acetate for 50 days prior to mating. Deficits in AAL were demonstrated in rats exposed to Pb either during pre-weaning or pre- and postweaning, creating a blood Pb level of 15 µg/dL and a brain Pb level of 0.09 to 0.16 µg/g wet weight. Rats that received only postweaning exposure (blood Pb of 16 µg/dL and brain level of 0.09 µg/g) had reduced deficits in AAL. Another study of AAL (Chen et al., 1997a) used SD rats exposed to 0.2% Pb acetate either during gestation and lactation (gestation day to until PND 21), during postweaning only (PND 21 until testing at PND 56), or continuously. Blood Pb levels at PND 56 were <2, 3.8, 25.3, and 29.9 µg/dL, respectively. No Pb-associated effects in learning were seen with just maternal or postweaning exposure. Compared to controls, continuously exposed rats showed a tendency of lower avoidance and higher no response levels in the two-way active avoidance tasks. Chen et al. (2001) tested step-down passive avoidance learning in SD rats similarly exposed. All three Pb-treated groups tested at PND 55 to 56 demonstrated impaired learning but unimpaired retention. Results of parallel autoradiographic analyses suggested that the Pb-induced deficits in acquisition were associated with alterations in AMPA receptor binding.

Open Field Performance

Salinas and Huff (2002) compared learning in Pb-exposed spatially trained and cue-trained F344 rats using an open field arena. The chronically exposed rats were tested at ~29 weeks of age when blood Pb levels were ~42 µg/dL. The Pb-treated rats trained to find food using extra-maze spatial cues demonstrated better performance than either controls or the Pb-exposed rats trained using intra-maze discrete cues. Additionally, by the seventh day of testing, both groups of Pb-treated rats spent less time on the periphery of the maze. The authors (a) hypothesized that "a Pb-induced overflow of mesolimbic DA may have facilitated the expression of rearing behaviors," which assisted in the spatial learning and (b) suggested that this overflow may cause "impulsivity" that results in less time spent by the Pb-treated rats on the periphery.

Effects of Pb on Memory

Studies reported in the 1986 AQCD showed that monkeys with steady state blood Pb levels of 33 µg/dL and tested at 3 to 4 years of age had impaired delayed matching-to-sample in both spatial- and nonspatial-based paradigms. However, Cory-Slechta (2003) pointed out that much of this work was done with animals that had previous behavior testing done with them and that may have altered results. New studies characterizing Pb-induced deficits in matching-to-sample tests have not been found. Other studies evaluating Pb-induced deficits in memory are described below.

Morris Water Maze

The Morris water maze tests both learning and memory by requiring rats to learn and remember the position of a platform hidden in opaque water. The effect of chronic developmental Pb exposure on water maze performance was tested in LE rats (Jett et al., 1997). Dams were dosed with 250-ppm Pb acetate in feed from 10 days before breeding through lactation. Offspring continued on the same Pb-dosed chow through testing. Blood Pb levels were not reported. Hippocampal Pb levels were 1.73 (PND 21), 1.02 (PND 56), and 0.91 µg/g wet weight (PND 91). Reference (long-term) memory and working (short-term) memory were tested on PND 21, 56, and 91, with different rats in each test. Pb-exposure had no effect on working memory at any age tested, but did affect reference memory (significant in females and nearly significant in males) in the PND 21 rats. This group (Jett et al., 1997) also demonstrated an increase in escape latency in adult LE rats injected with Pb acetate directly into the dorsal hippocampus. A study examined the effects of timing of Pb exposure (Kuhlmann et al., 1997) in which rats were exposed to 750 ppm Pb acetate in diet either maternally (gestation and lactation), permanently (gestation onward), or postweaning (at 750 or 100 ppm). Blood Pb levels at PND 100 were 1.8, 21.3, 22.8, and 26.3 µg/dL, respectively. Compared to controls, maternal and permanent exposure groups were impaired in water maze performance, with maternal exposure producing both the greatest escape latency and longest escape path length. There were no effects on performance in the postweaning exposure groups. This study provides evidence that early exposure can produce long-term deficits in learning and memory.

Yang et al. (2003) exposed Wistar rats gestationally to 0.03% (low), 0.09% (middle), or 0.27% (high) Pb acetate in food. Pups were fostered by control dams. Blood Pb levels were

~30, ~33, and ~42 μg/dL, respectively at PND 0 and ~2 μg/dL in all treatment groups at testing (PND 49). By measuring swim path and time spent in target quadrants, it was shown that Pb exposure at all three doses impaired memory retrieval in males. In female offspring, only the low dose affected memory retrieval, suggesting a greater impact of low level Pb exposure on females. Results on the fixed location/visible platform tasks showed that motor performance and vision were not affected by Pb treatment and suggested to the authors that gestational exposure is sufficient to cause memory deficits in young adults.

Results from recent studies have provided evidence that environmental enrichment during development may protect against Pb-induced effects on learning and memory deficits. Schneider et al. (2001) exposed male LE rats to 0 or 0.2% Pb acetate from PND 25 until later testing at PND 100. At PND 25, some of the rats were raised in isolation with no access to any stimulus objects, while the "enriched" rats were raised in groups of 8 with stimulus objects or toys. Blood Pb levels were ~5 μg/dL in controls and ~30 in Pb-treated animals. Pb-exposed rats raised in isolation demonstrated spatial learning deficits in the Morris water maze, whereas the Pb-exposed rats raised in the enriched environment performed better than the isolated Pb group. Additionally, Pb-exposed rats had diminished hippocampal levels of the neurotrophic factors brain-derived neurotrophic growth factor (BDNF), nerve growth factor-β, neurotrophin-3, and basic fibroblast growth factor. This suggested to the authors a possible relationship between Pb levels, neurotrophic factor levels, and diminished hippocampal development and function. Earlier exposures to Pb showed some similar effects of environmental enrichment (Guilarte et al., 2003); LE rats were exposed during gestation and lactation to 0- or 1500-ppm Pb acetate. Pb exposure was stopped at PND 21 and rats were placed in isolation or in an enriched environment. At PND 50 when blood Pb levels were 0.25 and 3.9 μg/dL, respectively, testing in a water maze showed enhanced performance of the Pb-treated rats raised in the enriched environment. The environmental enrichment was accompanied by increased gene expression in the hippocampus of NMDA receptor subunit 1 and BDNF. These results demonstrate that Pb-induced learning impairments and molecular changes in hippocampus can be reversed by environmental enrichment, even after the exposure has occurred.

Delayed Alternation

Delayed alternation trials assess both memory and attention by requiring the animal to alter a previous response following a delay period. Typically, the longer the delay between choices, the greater the inaccuracy of the choice. A number of studies have examined Pb effects on delayed alternation tasks in rodents. To compare the effects of Pb exposure on memory during different stages of life, rodent studies of this endpoint were completed (Cory-Slechta et al., 1991). PND 21 (young), 8-month-old (adult), and 16-month-old (old) F344 rats were exposed to 0, 2, or 10 mg/kg Pb acetate, resulting in blood Pb levels of ~23 at the 2 mg dose and of ~42 (adult), ~48 (old), and ~58 µg/dL (young) at the 10 mg dose. Training began after 4 months of exposure and testing continued until 8.5 months of exposure. Rats were trained with many sessions using a cue light alternating between two positions and tested after 4 months of exposure. Aging itself caused impaired accuracy. In both young and old rats, Pb exposure increased accuracy, at the longest delay periods (12 sec) in young rats and at the short delay periods in old rats. In adult rats, performance was not affected by Pb exposure. The authors hypothesized that the improved performance was due to perseveration of alternation behavior learned during the training and that both young and old animals may have enhanced vulnerability to Pb. The effect of chronic postweaning Pb exposure (0, 75, or 300 ppm in drinking water starting at PND 25) on DSA was evaluated in LE rats (Alber and Strupp, 1996). Blood Pb levels were 19 and 39 µg/dL. At 22 weeks of age, nose poke training was followed by cued alternation, spatial, and DSA training. Learning the alternation rule was not affected by Pb treatment. Across all delay periods, the treated rats performed more poorly than controls, suggesting that memory was not affected by Pb exposure. An additional finding was that Pb induced side bias, a strategy commonly adopted by rats in response to an insoluble problem.

Nonhuman primate DSA studies have also been completed. Levin and Bowman (1986) evaluated DSA in 5- to 6-year-old rhesus monkeys exposed to two early pulses of 10 mg/kg Pb acetate and a chronic exposure of 0.7 mg/kg/day for the first year. The pulses of Pb were done to simulate brief periods of higher-level exposure that can occur in children with Pb pica. Peak blood Pb levels were 250 to 300 µg/dL, which decreased to 80 µg/dL for the remainder of the first year. Deficits occurred most commonly with short inter-trial delays, suggesting that memory was not affected by Pb, but that deficits in attention or strategy may have been present. Most Pb-induced deficits were accounted for by lose-shift errors, possibly due to perseveration

on the alternation strategy despite loss of reinforcement. The authors hypothesized a "window of sensitivity" to Pb, wherein exposures during the first year of life can create long-term cognitive deficits. Another cohort of rhesus monkeys was dosed with 1.0 mg/kg/day Pb acetate for the first year of life, producing a blood Pb level of 70 µg/dL (Levin and Bowman, 1989). They were tested on DSA at 4 years of age, by which time blood Pb levels had returned to control levels. The monkeys performed better than controls on choice accuracy, which suggested to the authors that a Pb-induced decrease in attentiveness may make the monkeys less susceptible to irrelevant stimuli and thus able to perform better.

Rice and Karpinski (1988) evaluated the effects of low-level lifetime exposure on delayed alternation tasks. Cynomolgus monkeys were dosed with 0, 50, or 100 µg/kg/day Pb acetate from PND 1 through the completion of the study. Blood Pb levels peaked at PND 100 (~3, 15.4, and 25.4 µg/dL, respectively) and reached steady state by PND 300 (3, 10.9, and 13.1 µg/dL). When tested at 7 to 8 years of age, using delay values that increased over the course of the trial, Pb-induced impairment of initial acquisition of tasks was observed. Longer delays between alternations resulted in poorer performance by the Pb-treated monkeys and perseverative behavior, sometimes lasting for hours. Further, the treated monkeys repeatedly pounded buttons indiscriminately, suggesting to the authors a failure to inhibit inappropriate responding. Rice and Gilbert (1990b) attempted to evaluate the effect of timing of exposure on this endpoint. The cynomolgus monkeys described above (Rice and Gilbert, 1990a) that were exposed to Pb either continuously from birth, during infancy only, or beginning after infancy were given a set of DSA tasks at 7 to 8 years of age. Monkeys had to push two buttons alternately with delays increasing from 0.1 to 15 sec. All Pb-treated groups had the same impairments of initial acquisition, indiscriminate responding, greater impairment with longer delays, and perseverative responses. All three exposure groups had similar degrees of impairment, indicating to the authors a possible lack of sensitive period for Pb's affects on this endpoint.

Levin et al. (1987) examined potential involvement of cholinergic (ACh) and DA neurotransmitter systems in Pb-induced impairments of DSA performance. Rhesus monkeys were exposed during the first year of life to daily doses of 0.7 mg/kg/day plus two early high pulses (10 mg/kg/day during the second and fourth weeks of life). Blood Pb levels were 63 at weeks 1 to 4, 174 at weeks 5 to 10, 68 at weeks 11 to 52, 4 at 6 years of age, and 2 µg/dL by the time of testing (7 to 9 years of age). Thirty min prior to testing, one of the following drugs was

administered: the DA receptor blockers, haloperidol and sulpiride; the muscarinic ACh receptor blocker, scopolamine; or the DA agonist, amphetamine. In addition, amphetamine and L-dopa were injected for 2 weeks for chronic testing. In both controls and Pb-treated monkeys, scopolamine caused a dose-related decline in performance. Chronic L-dopa ameliorated the Pb-induced DSA deficits, which returned following cessation of L-dopa administration. These data again show long-term cognitive impairments resulting from early Pb exposures and implicate DA mechanisms as a causal factor in these impairments.

Avoidance

A shuttle avoidance task evaluated retention in rats exposed during gestation and lactation to 0.5, 2.0, or 4.0 mM lead acetate in drinking water (Rodrigues et al., 1996a). Blood Pb levels ranged from 11 to 50 µg/dL. The Pb-treated rats demonstrated no increases in avoidance response between sessions, suggesting less retention in the treated animals compared to controls. Murphy and Regan (1999) exposed Wistar rats from PND 1 to PND 30 to 400 mg of Pb chloride/L drinking water, producing blood Pb levels of 10 to 15 µg/dL by PND 8, ~45 µg/dL by weaning, and 2 to 4 µg/dL by PND 80. At PND 80, the rats were trained on a 1-trial, step-through, light–dark passive avoidance test. At 48 h postexposure, the rats showed no Pb-induced changes in recall; but at 5 days postexposure, the rats exhibited a decline in recall. The authors hypothesized that the Pb exposure affected long-term memory storage.

Discrimination Retention

Munoz et al. (1986) used visual discrimination learning and spatial learning in a retesting approach to evaluate changes in long-term memory storage in Wistar rats. The rats were exposed to 750-ppm Pb acetate either through PND 16 (maternal exposure) or chronically through testing. Blood Pb in males tested at PND 110 was <1 µg/dL in controls and maternally exposed rats and 34 µg/dL in chronically exposed rats. Males were tested at PND 100 in visual discrimination and then retested 42 days later. Both Pb-treated groups learned the original discrimination comparably to controls, but both groups showed a deficit in retention of the discrimination. Females were tested at PND 180 in spatial discrimination, then retested 4 weeks later. The Pb-treated female rats took longer to reach criterion in the acquisition learning and longer to eat the pellets in the retention phase. These data suggest that early gestational Pb

exposure can cause long-lasing retention deficits and that subsequent direct dietary exposure does not potentiate these effects.

Effects of Pb on Attention

Human studies have suggested that Pb exposure is associated with deficits in attention (e.g., inattentiveness, impulsivity, distractibility, attention deficit disorder); however, only scant evidence from early animal studies pointed to attention deficits as a causal factor in neurocognitive dysfunction. Related to deficits in attention are increased response rates and response perseveration, both of which are associated with Pb exposure as discussed above. Several laboratories have examined the effect of Pb on attention specifically. Brockel and Cory-Slechta (1998) exposed male LE rats to 0-, 50-, or 150-ppm Pb acetate in water from weaning. After 3 months of exposure, blood Pb levels were <5, 10.8, and 28.5 µg/dL, respectively. After 40 days of exposure, the rats were trained on a FR/waiting-for-reward behavioral baseline, learning to produce food delivery by pressing a lever 50 times. Additional food could be earned by withholding lever presses (i.e., by waiting); free food was given at increasing time intervals after completion of the FR. In the FR component of the study, the high-dose animals had significantly higher response rates and more frequent resets of the waiting period than the low dose group and controls. Wait time was significantly lower in both treated groups compared to controls in the waiting behavior component. The high-dose animals also had an increased number of reinforcers and a higher response to reinforcement ratio than low dose and controls. These data suggest that blood Pb levels as low as 11 µg/dL are associated with inefficient response patterns and an inability to manage delays of reinforcement. The authors hypothesized that this pattern of behavior in humans could have the consequence of eventual dissipation of effort or lack of motivation.

Involvement of dopamine-like receptors in the Pb-induced decrements in waiting behavior was tested using this FR/waiting-for-reward schedule (Brockel and Cory-Slechta, 1999a). The same 0, 50, and 150 Pb dosing was done, resulting in blood Pb levels of <5, 9.7, and 26.2 µg/dL after both 3 and 7 months of exposure. Following performance stabilization, drugs were administered IP 30 min prior to behavioral testing: the D_2 agonist quinpirole, the D_1 agonist SKF 82958, the D_2 antagonist eticlopride, or the D_1 antagonist SCH 23390. The drugs administered to control rats did not cause Pb-like effects. All the drugs decreased FR response

rates, but only quinpirole reversed the Pb-induced effects on FR response rate, FR resets, wait reinforcers, and wait time. This suggested to the authors a role for D_2 receptors in Pb's effects, a dissociation of Pb's effects on FR and waiting time, and a possibility that decreased waiting behavior is a direct effect of Pb exposure.

A similar postweaning Pb exposure resulting in blood Pb levels of <5, 16.0, and 28.0 µg/dL was used to evaluate sustained attention (Brockel and Cory-Slechta, 1999b). Food rewards were obtained by the rats for discriminating correctly between a target and distracter light. A 13-sec time-out was given for incorrect responses. Lead produced no effects on sustained attention despite broad modifications of many parameters of the task, suggesting to the authors that Pb affects other aspects of attention and that Pb-induced attention deficits are modified by time-out contingencies and reinforcement. Morgan et al. (2001) evaluated changes in attention in a study with LE rats exposed to Pb acetate in drinking water. One group (GL300) received Pb throughout gestation and lactation (GL), other groups received 300 or 600 ppm Pb during lactation only (L300 and L600). Blood Pb levels were <5 for controls, 36 to 43 at PND 8, 27 to 34 at PND 24, 131 to 158 at PND 53, and 16 to 18 µg/dL at PND 53 (treated animals). Lead-exposed animals (both GL and L) committed more errors of omission when a delay was imposed prior to cue presentation and in trials that followed an incorrect response. Response initiation was also impaired in Pb-treated animals in a sustained attention task in which the onset and duration of the visual cue varied randomly across trials. These data suggested to the authors that early Pb exposure caused long-lasting increased reactivity to errors and impairment of sustained attention. Inconsistencies in these results compared to those of Brockel and Cory-Slechta (1998,1999b) may be accounted for by the differences in exposure period and the higher blood Pb level and the small percentage change in endpoints in the later study. In a review of the attention literature, Cory-Slecta (2003) stated that impulsivity and waiting-for-reward behavior may be more strongly affected by Pb than are sustained attention and hyperkinesis. Further, the Pb-induced impulsivity as a behavioral dysfunction may lead to cognitive impairments.

Effects of Pb on Motor Function, Locomotor Activity, and Vocalization

Evaluations of the effects of Pb on development of motor function and reflexes discussed in the 1986 AQCD showed that Pb affects the air righting reflex in rat pups with blood Pb levels of 35 µg/dL and rotarod performance at 175 µg/dL. Developmental lags in gross activity were

produced in rat pups with blood Pb as low as 14 μg/dL. Studies ruled out the possible contribution of Pb-exposed dams to their offspring's slowed development. In the early evaluations of spontaneous activity, numerous issues were apparent, including a lack of consensus for the definition of "activity" and activity being affected by confounding variables such as age, sex, estrous cycle, time of day, novelty of environment, and food deprivation. Discrepant findings summarized in the 1986 Lead AQCD included 11 studies showing increased activity with Pb exposure, 6 studies showing decreased activity, and 28 studies showing age-dependent, qualitative, mixed or no changes. Thus, no conclusions were reached regarding Pb effects on these endpoints.

Lilienthal et al. (1986) evaluated activity in the rhesus monkeys exposed prenatally to Pb as discussed above. Activity, measured in unfamiliar environments at age 12 to 15 months, showed no Pb-related effects in these monkeys. In the study of schedule-controlled behavior discussed above, Newland et al. (1996) identified a deficit in the physical execution of pulling a weighted bar in Pb-treated monkeys, suggesting a motor impairment occurring long after the exposure period. Open field behavior was assessed in rhesus monkeys exposed to a "pulse-chronic" dosing paradigm (two pulses of 10 mg/kg the first month of life and chronic level of 0.7 mg/kg/day for the rest of the first year of life) (Ferguson and Bowman, 1990). Blood Pb levels peaked at 5 weeks of age (55 μg/dL), averaged 36 μg/dL for the remainder of the first year, and were ≤5 μg/dL for at least 1.5 years prior to testing at 4 years of age. The Pb-treated monkeys demonstrated a longer latency to enter the open area, increased durations of activity and environmental exploration, as well as a failure to habituate.

Laughlin et al. (1999) evaluated the effects of Pb on behavior of neonatal rhesus monkeys using the Schneider Neonatal Assessment for Primates (SNAP). Monkeys were dosed orally with Pb acetate daily to eventually achieve a target blood Pb level of 35 μg/dL. Blood Pb levels for controls was <5 μg/dL, and for treated animals was 15 to 20 μg/dL during week 3 and 22 to 28 μg/dL during week 4. Testing was done during the first four weeks of life, at which time few differences between control and Pb-exposed monkeys were seen. The authors reported less stability in SNAP performance in the Pb-exposed monkeys compared to controls, which they suggested may be caused by a disruption of continuity of development by Pb. The same animals were evaluated for exploration behavior starting at the second postnatal week (Lasky and

Laughlin, 2001). The Pb-exposed monkeys demonstrated more agitation, climbing, fear, and exploration of the periphery than controls.

In the Rodrigues et al. (1996a) study cited above, rats were also subjected to open-field testing, where a Pb-associated increase in locomotor activity was observed. To assess the effects of developmental exposure on a number of neurobehavioral endpoints, Wistar rats were exposed during gestation and lactation to 500-ppm Pb acetate (Moreira et al., 2001). Blood Pb levels were 41 µg/dL (dams), 21 µg/dL (PND 23 pups), and <0.1 µg/dL (PND 70). The PND 23 pups exhibited Pb-induced increased ambulation in the open-field tests, decreased exploratory behavior in the holeboard tests, and no differences from control in the elevated maze tests. The PND 70 rats showed a Pb-induced increase in head dipping in the holeboard test. No differences were noted in the rotarod tests. Ferguson et al. (1998) evaluated activity in SD rats exposed to 350-ppm Pb acetate (through dams' drinking water) from birth until weaning. Blood Pb was 46 µg/dL in pups at weaning. No Pb-related effects were seen in the following behavioral assessments: play (PND 38 and 45), burrowing (PND 49–54), dominance (PND 58 and 65), residential running wheel (PND 67–80), residential figure 8 maze (PND 70–84), complex maze (PND 83–94), acoustic startle (PND 98), emergence (PND 121 or 128), and prepulse inhibition (PND 177). The authors suggested that at these Pb exposure levels, the development of Pb-induced functional changes may require substantial demands on the system for detection.

To evaluate the effects of early Pb exposure on activity and vocalization, De Marco et al. (2005) exposed female Wistar rats to 8, 16, or 24 mg/mL lead acetate in water, allowed them to breed, and then crossfostered pups to them to allow exposure during pregnancy, during pregnancy and lactation, or during lactation. In the treated rats, blood Pb levels ranged from 5.7 to 36.6 µg/dL, with levels dropping to 0.5 µg/dL in adults. In all three exposure groups, the PND 7 pups showed a dose-dependent decrease in ultrasonic vocalization, whereas the PND 14 pups showed an increase compared to controls. These results contrast with the normal developmental pattern of vocalization and suggested to the authors a Pb-induced alteration in the maturation pattern of this behavior. Additionally, the PND 14 pups showed higher activity levels than controls.

One animal study has shown behavioral deficits in offspring resulting from paternal exposure to Pb (Nelson et al., 1997). Male Dutch Belted rabbits were dosed with Pb acetate for

15 weeks to produce blood Pb levels of <5 (control), 20, 40 and 80 μg/dL. They were then mated with nonexposed females and the offspring were tested for exploratory behavior at PND 15, 20, 25, and 30 in a figure-eight activity monitor. For those F2 offspring of male rabbits that had paternal blood Pb ≥40 μg/dL, exploratory behavior was affected at PND 25, the time of peak activity in rabbits. This is the only animal study available showing these effects. Another study demonstrated that behavioral effects of Pb can extend to a second generation (Trombini et al., 2001). First, Pb-induced behavioral effects were assessed in Wistar rats exposed during gestation and lactation. Pregnant rats were dosed with 750 ppm lead acetate in drinking water. blood Pb levels in offspring were 25 μg/dL at PND 30 and 0.1 μg/dL at PND 90. No Pb-associated changes in elevated maze behavior were noted in 30- and 90-day-old rats. In open-field behavior studies, PND 30 Pb-treated rats showed decreased freezing, increased ambulation, and increased grooming. PND 90 Pb-treated rats showed decreased freezing and increased ambulation. Offspring of Pb-treated females were mated with nonexposed males and evaluated at PND 30 and 90. At both ages, the F2 generation rats demonstrated increased ambulation and decreased grooming, suggesting evidence of intergenerational effects of Pb.

In an attempt to discern the mechanism of motor deficits resulting from Pb exposure, Morley et al. (2003) exposed Drosophila instar larvae to 100 μM Pb acetate and examined the neuromuscular junction. They observed nonuniform matching between the size of the motor terminal and the muscle area, suggesting a possible mechanism of Pb's effects on motor function.

Effects of Pb on Social Behavior

Early work evaluating Pb-induced alterations in social behavior or behavioral interactions showed inconsistent results. In both rats and monkeys, Pb tended to reduce aggressive behavior. Pb-treated mice demonstrated gender differences in sexual interaction and social investigation, which could have been attributed to differences in brain Pb concentrations. Interactions between mothers and offspring were shown to be affected by Pb exposure. These included increased clinging by infants, less food-seeking activity by pups, suppressed play behavior, increased time spent in the nest by dams, and increased retrieval of pups to the nest by dams.

Studies published since the 1986 Lead AQCD have examined the effects of Pb on social behavior in greater detail. Donald et al. (1986) exposed male and female BK:W mice to

0.13% Pb acetate in drinking fluid before breeding. At weaning, the pups were continued on the 0.13% Pb chronically. At 18 weeks, brain and femur Pb concentrations in males were 27.6 and 998 μM Pb/g ash, respectively (controls were 7.5 and 78). At 34 weeks, brain and femur concentrations in males were 445 and 5364 μM Pb/g ash (controls 40 and 100). At 34 weeks, brain and femur concentrations in females were 787 and 4026 μM Pb/g ash (controls 88 and 334). Blood Pb levels were not reported. Young Pb-exposed female mice habituated more slowly to the environment, while young Pb-exposed males habituated more rapidly. In general, in adults, Pb caused an enhancement of social and sexual investigation. The same laboratory (Donald et al., 1987) evaluated activity resulting from a higher Pb exposure (0.25% chronically in drinking fluid from conception). Exploratory behavior and social investigation were increased in both sexes of Pb-treated mice at age 3 to 4 weeks compared to controls. At age 7 to 8 weeks, social investigation was increased, but exploratory behavior was decreased in Pb-treated mice. At age 15 to 16 weeks, nonsocial activity was decreased in females, but increased in males. At age 17 to 18 weeks, Pb-treated males demonstrated shorter latencies to aggression than controls.

Holloway and Thor (1987) tested social behavior in LE rats exposed to 500-ppm Pb chloride during lactation. They estimated blood Pb to be 42 μg/dL on PND 20 based on similar exposures. AT PND 11, they found that Pb induced no sex differences, no effects on pup activity, and no differences in pup retrieval by dams. At PND 26, Pb treatment influenced all social behavior tested (i.e., investigation duration and frequency, crossover frequency, pinning) but did not change activity levels compared to controls. At PND 36, Pb-treated pups demonstrated increased crossover frequencies but no change in activity levels compared to controls. The authors hypothesized that low-level Pb exposure increases social investigation and "rough and tumble" play behaviors, due to increased behavioral reactivity to stimuli, but does not increase aggression. Pb-induced changes in aggression were assessed in golden hamsters exposed to 100 ppm lead acetate from gestational day 8 until PND 42 (Delville, 1999). Blood Pb concentration at PND 42 was 10 to 15 μg/dL. At PND 19 to 20, the Pb-exposed hamsters were significantly smaller than controls and exhibited less play fighting. At PND 45, the treated and control animals' weights were not significantly different, and the treated animals displayed more aggression as measured by attacking and biting an intruder put in the cage. In the assessment of developmental Pb exposure discussed above (Moreira et al., 2001), the early Pb exposure resulted in a decrease in social interaction time in the PND 70 rats.

Levin et al. (1988) exposed rhesus monkeys to a pulse-chronic Pb exposure protocol as described above (Levin and Bowman, 1986) resulting in a blood Pb level of 56 µg/dL during week 5 and 33 to 43 µg/dL for the remainder of the first 6 months of life. During the first 6 weeks after birth, results of the Early Infant Behavioral Scale showed Pb-induced lowered muscle tonus and greater agitation, but no effects on sensorimotor measures. Beginning at PND 14, monkeys were tested on a Piagetian object permanence task, which revealed no Pb-related effects. Starting at 2 months of age, monkeys were tested on a visual exploration task, which showed decreased visual attentiveness in Pb-treated monkeys. Laughlin et al. (1991) evaluated the effects of Pb exposure and diet in rhesus monkeys exposed to 1 mg/kg/day Pb acetate from PND 5 until PND 365. Monkeys were given either low-milk or high-milk diets because of milk's ability to enhance tissue levels of Pb. Blood Pb levels reached a plateau of ~70 µg/dL during the first year when initial testing occurred and then decreased to ~ 35 µg/dL at 16 months postexposure. During the first year of life, Pb-induced disruption of social play and increases in both self-stimulation and fearful behavior were observed. At 16 months of age, these changes were still present. Differences in milk intake had little effect on behavior in this study.

Pb Exposure and the Stimulus Properties of Neuropharmacologic Agents

The drug discrimination paradigm has been utilized to characterize postsynaptic receptor status for multiple neurotransmitter systems. Rats chronically exposed to Pb beginning at weaning and tested as adults were trained to discriminate either a systemically administered D_1 or D_2 receptor agonist (Cory-Slechta and Widzowski, 1991). Exposed rats learned the discrimination task more rapidly than controls and exhibited greater levels of responding to lower doses of the training drugs and less blockade by a D_2 receptor antagonist, consistent with generalized dopaminergic receptor supersensitivity. In groups of animals exposed only from birth to weaning and trained to discriminate the same drugs, the D_2-D_3 subtype receptor supersensitivity in exposed animals was again present, but no changes in responding to the D_1 agonist were apparent (Cory-Slechta et al., 1992). Further work with this test employing the postweaning exposure protocol failed to demonstrate any D_1-D_2 receptor interactions in the supersensitivity displayed by Pb-exposed animals (Cory-Slechta et al., 1996a).

To test cholinergic sensitivity in animals chronically exposed after weaning, rats were trained to discriminate a muscarinic agonist (Cory-Slechta and Pokora, 1995) and were tested in the added presence of a muscarinic antagonist. The results suggest an increased sensitivity to at least one subtype of muscarinic receptor in Pb-exposed rats.

Glutamatergic functioning also has been assessed by use of the drug discrimination paradigm. Rats chronically exposed beginning at weaning and tested as adults exhibited diminished responsiveness to an NMDA subtype receptor antagonist (Cory-Slechta, 1995), but enhanced responding to lower doses of NMDA (Cory-Slechta et al., 1996a). When exposure was limited to the period between birth and weaning, the diminished sensitivity to the NMDA receptor antagonist was less evident but still present (Cory-Slechta, 1997).

Thus, the drug discrimination method appears to have provided useful insights into the status of some neurotransmitter systems in chronically exposed animals. The reports cited above indicate an upregulation of dopaminergic, cholinergic, and glutamatergic receptors that are generally consistent with findings of diminished presynaptic function described earlier in this section. Nonetheless, this paradigm has some limitations. As all drugs in the cited studies were administered systemically, the results provide no evidence on brain regional sites of action. In addition, the chronic intermittent administration of the training drug has the potential to induce compensatory neuronal changes by itself and may thusly mask or otherwise alter the manifestation of the effects of Pb exposure.

5.3.6 Lead-Induced Changes in Cellular Development and Disposition of the Metal

Alterations in cellular differentiation and morphology can be important structural components of the manifestations of Pb neurotoxicity in neurons and glia. While these issues have not been thoroughly addressed by research investigations, important observations have nonetheless been made, as discussed below in the following subsections.

Lead Exposure and Neural/Glial Progenitor Cells

Recent Pb neurotoxicity studies have evaluated the effects of Pb exposure on neural and glial progenitor cells. Chronic Pb exposure of rats, beginning at PND 25 and producing blood Pb levels of 20 μg/dL at the termination of exposure, was found to significantly decrease

proliferation of new cells in the dentate gyrus compared to control animals (Schneider et al., 2005). Other workers determined that continuous exposure from birth to adulthood producing blood Pb levels of 35 to 40 µg/dL reduced the total number of labeled cells in the hippocampal dentate gyrus at 28 days after the last administration of a DNA synthesis marker (Gilbert et al., 2005). Rats whose exposure was terminated at weaning showed no changes in cellular labeling or survival, indicating that chronic exposure reduces the capacity for hippocampal neurogenesis.

Studies have also been conducted to investigate the effects of Pb exposure on glial progenitor cells. Deng et al. (2001) examined cultured oligodendrocytes and their progenitor cells acutely exposed to Pb^{2+} in vitro; they observed an exposure-induced delay in the differentiation of the progenitors and that the progenitor cultures were more sensitive to Pb^{2+} than the mature oligodendrocytes. These findings suggested interference with the timely developmental maturation of the progenitor cells. A subsequent study found that a low concentration of Pb^{2+} in vitro inhibited proliferation and differentiation of these progenitors without affecting cell viability (Deng and Poretz, 2002). Proliferative capability was decreased and cell-intrinsic lineage progression was inhibited at a late progenitor stage. Thus, acute Pb^{2+} exposure suppresses both the proliferation and differentiation of progenitor cells.

Lead Exposure and Neurite Outgrowth

Neurite initiation is highly sensitive to neurotoxic compounds and has been the focus of studies examining morphological alterations caused by in vitro exposure to Pb^{2+}. Kern and Audesirk (1995) found that 100 nM Pb^{2+} inhibited neurite initiation in cultured rat hippocampal neurons and, on the basis of results with kinase inhibitors, concluded that this occurred by inappropriate stimulation of protein phosphorylation by Ca^{2+}-calmodulin-dependent or cyclic AMP-dependent protein kinases, possibly through stimulation of calmodulin. Intracellular free Ca^{2+} concentrations were not altered by up to 48 h exposure to nominal 100 nM Pb^{2+}, suggesting that the stimulation of the above kinases or calmodulin were not via increased Ca^{2+}, but instead were attributable to intracellular Pb^{2+} concentrations. Evidence of Pb^{2+}-induced inhibition of neurite outgrowth is in general agreement with results seen after chronic exposure to Pb employing in vivo models. Cline et al. (1996) employed an exposure protocol of 0.1 nM to 100 µM nominal Pb^{2+} for 6 weeks localized to the retinotectal system of frog tadpoles; they observed that the area and number of retinal ganglion cell axon arborizations within the optic

tectum was reduced at nanomolar Pb^{2+} concentrations. As discussed in Section 5.3.4, Reuhl et al. (1989) exposed primates to 2 mg Pb/kg/day from infancy to 6 years of age and found that neuronal volume density was reduced in primary visual area V1 and in visual projection area V2, compared to a group exposed to 25 μg Pb/kg/day. Moreover, a relative decrease in the number of arborizations among pyramidal neurons in both areas V1 and V2 was observed in the higher-dose group. Thus, there was good correspondence between reports that acute Pb^{2+} exposure in vitro and extended exposure in animal models in vivo results in diminished neuronal growth and differentiation at Pb levels of apparent environmental relevance. Studies employing intact animals have not investigated specific cellular mechanisms underlying these effects.

Lead Exposure and Neural Stem Cells

Given considerable contemporary interest in the use of neural stem cells to treat various neurological diseases, the efforts of Huang and Schneider (2004) to examine the actions of exposure to Pb^{2+} in vitro on these cells is noteworthy. Pb exposure produced no effect on neurosphere viability, but caused a significant dose-dependent inhibition of proliferation. In addition, the number of neurons differentiated from Pb^{2+}-exposed neurospheres was significantly decreased versus control, as were the number of oligodendrocytes obtained. However, Pb exposure increased the number of astrocytes obtained. These observations suggest an important Pb^{2+}-induced influence on stem cell proliferation and differentiation.

Lead and the Blood-Brain Barrier

Early work demonstrated that the capillary epithelium in the brain is a target for Pb and that Pb intoxication can disrupt the blood-brain barrier (BBB). Pb-exposed capillary endothelial cells isolated from rat cerebral cortex showed deposits of Pb preferentially sequestered in mitochondria, suggesting Pb-induced disruption of transepithelial transport of Ca^{2+} and other ions. Furthermore, the developing CNS is especially sensitive to Pb-induced vascular damage. Cerebral endothelial cells are known to accumulate Pb much more than other cell types and the choroid plexus in both humans and animals accumulates much higher Pb concentrations than other brain regions. However, these studies employed high exposure levels and, thus, are of limited utility in evaluating the effects of environmentally relevant exposures.

Bradbury and Deane (1986) examined the rate of uptake of ^{203}Pb into brain and other soft tissues of the rat at constant radiotracer levels in plasma. Uptake of ^{203}Pb in the brain was linear up to 4 h, and the authors hypothesized that the capillary endothelium was the rate-limiting component of Pb transport into the brain because of its relatively small area. The rate of uptake in the brain contrasted with that into cisternal cerebrospinal fluid, which plateaued at ~5% of that of plasma at 1 to 2 h, and into the choroid plexus, in which Pb accumulated to ~350% of plasma levels at 4 h suggesting a 10-fold greater uptake. Bradbury et al. (1991) found that albumin rarely enters the brain from blood, suggesting that Pb transport into brain is likely as free Pb^{2+} or Pb in the form of inorganic complexes (e.g., $PbOH^+$, $PbHCO_3^+$, or $PbCl^+$) or Pb bound to a low molecular weight organic ligand (e.g., cysteine). Further work (Bradbury and Deane, 1993) using short vascular perfusion of a cerebral hemisphere determined that ^{203}Pb enters the brain very quickly in the absence of an organic ligand but that transport is abolished in the presence of albumin, L-cysteine, or EDTA. It was proposed that $PbOH^+$ or some other simple organic Pb^{2+} complex passively enters the endothelium, and that the entry is mitigated by active back transport of Pb^{2+} into blood by Ca-ATPase pumps.

To evaluate mechanisms by which Pb increases the permeability of the BBB, Dyatlov et al. (1998a) dosed BALB/cByJ suckling male mice with 2.5 µg/g body weight of Pb acetate, LPS (100 ng/g body weight), recombinant IL-6 (5 ng/body weight), Pb + IL-6, or sodium acetate + LPS. Following five injections over 10 days, they measured the transendothelial electrical resistance across the BBB. Pb at this level alone had no effect, but did potentiate the increases due to LPS. Pb plus IL-6 also caused a delay in the increase in arteriole resistance. Thus, Pb potentiates the actions of both IL-6 and LPS. Glutamate topically applied to the cerebrum caused a reversible decrease in resistance, whereas Pb caused this decrease to be irreversible. The authors hypothesize that this disruption of the BBB allows glutamate to enter the brain, further disrupting the BBB and irreversibly potentiating brain injury.

Lead also compromises the function of the barrier between the cerebrospinal fluid and systemic circulation, allowing transfer between tight junctions of the choroid plexus. Zheng et al. (1996) demonstrated that chronic Pb exposure (50 or 250 mg/mL in drinking water for 30, 60, or 90 days) reduced transthyretin levels in cerebrospinal fluid of male weanling rats in the presence of blood Pb levels of 18.2 and 48.9 µg/dL, respectively. Transthyretin, which is expressed in early fetal development, is produced in the choroid plexus and is the major thyroid

hormone binding protein, allowing transfer of thyroxine from choroid plexus into cerebrospinal fluid. The authors proposed that this Pb-induced reduction in transthyretin may be a factor in Pb-induced alterations in brain development.

Thus, the BBB allows significant entry of Pb into both the adult and developing brain. This transport is dependent on the chemical form of Pb, interactions of Pb with proteins and other components of the blood, and other biochemical and physiologic factors that are not fully defined. Information regarding Pb-binding proteins in brain are presented in Section 5.11.2.

Tissue Uptake of Lead

Pb^{2+} appears to be taken up into cultured cells by multiple ion channel-based mechanisms, including influx through channels activated by depletion of intracellular Ca^{2+} stores, non-L-type Ca^{2+} channels, and NMDA receptor-associated channels (Kerper and Hinkle, 1997; Mazzolini et al., 2001). Astroglia are well-known to act as Pb sinks and in culture and can accumulate up to 24 times more of the metal than neuronal cells (Lindahl et al., 1999). There is also evidence that glutathione may regulate Pb uptake into astroglia.

Histologic studies (e.g., Strużyńska et al., 1997) have demonstrated the transport of Pb into the brains of chronically exposed adult animals. Weanling rats were dosed with 2 g/L lead acetate in water for 3 months, creating blood Pb levels of 39 µg/dL. Using horseradish peroxidase as a tracer of vascular permeability, leaky microvessels were demonstrated by both light and electron microscopy. Focal leakage of tracer was observed in the short segment wall of microvessels, the surrounding neuropil, and regions of parenchyma near microvessels. Staining was also evident in the cytoplasm of pericytes and on the basement membrane of endothelial cells.

Accumulation of Pb in Blood and Brain

Early animal studies often neglected to include blood Pb and concomitant tissue levels achieved by the exposure protocols. The 1986 Lead AQCD was able to draw some limited conclusions about the relationship of exposure levels to blood and brain Pb concentrations. In general, at exposure concentrations of >0.2% Pb in drinking water and for exposure durations extending beyond the birth to weaning period, the ratio of blood to brain Pb concentration is

<1, suggesting that even as blood Pb peaks and then falls due to excretion or removal, the high-affinity binding of the metal to brain proteins promotes further accumulation in the brain.

The decline in blood Pb after exposure is terminated has been shown to depend on the level and duration of exposure (O'Flaherty et al., 1982; Hryhorczuk et al., 1985). Widzowski and Cory-Slechta (1994) exposed rat pups to various concentrations of Pb in the drinking water from birth to weaning, and they found that blood Pb declined rapidly with a half-life of <20 days. Wedeen (1992) reported a half-life of ~1 month in children, but Manton et al. (2000) found half-lives of 10 to 38 months in young children, depending somewhat on duration of Pb exposure from home remodeling. Because of the dependence of decreases in blood Pb concentrations on exposure level and duration, estimates of half-life vary significantly from one exposure scenario to another.

Using the same exposure protocol, Widzowski and Cory-Slechta (1994) measured brain regional half-lives for Pb and found that these values averaged ~20 days and did not vary between brain regions. Because of binding to brain tissue proteins, Pb concentrations in brain decline or respond to chelation more slowly than do blood Pb levels (Stangle et al., 2004). Other studies describing chelation of Pb are included in Table AX5-3.5.

5.3.7 Susceptibility and Vulnerability Factors Modifying Lead Exposure and Thresholds for CNS Effects

The effects of chronic Pb exposure may be modified by a number of physiologic variables and exposure parameters that can significantly enhance or diminish the toxic response. These susceptibility factors impact the toxic manifestations observed in the organism. A few of these factors are considered below.

Aging

The neurotoxic effect of chronic exposure to low level Pb with advancing age is becoming an important public health social issue (Yun et al., 2000a). Physiologic conditions associated with bone resorption, e.g., pregnancy, lactation, and aging, can potentiate the CNS effects of Pb and enhance exposure of adults. Such demineralization conditions can also add to the in utero exposure of the fetus, and postmenopausal resorption can increase PbB levels in women by 25% (Silbergeld, 1990).

Bone Pb levels serve as a marker of the burden of Pb in middle-aged and elderly men. During the demineralization that occurs during aging, blood Pb levels increase and may lead to declines in cognitive function (Weisskopf et al., 2004a,b; Payton et al., 1998). Supporting data have been obtained from animal studies. Cory-Slechta (1990b) administered various amounts of Pb for a constant duration beginning in young, adult, or old rats. An increased vulnerability to Pb was observed in older animals due to increased exposure from an elevated resorption from bone and an apparently greater sensitivity to the biochemical effects of the metal. Yun et al. (2000b) have presented evidence suggesting that this increased vulnerability may also be due to cerebral energy depletion in exposed animals. However, such findings have not been uniformly observed. Rice and Hayward (1999) exposed monkeys to Pb chronically and tested temporal visual function and contrast sensitivity at two different ages. Pb-exposed subjects exhibited differences in temporal function at the first, but not the second assessment, and there was no sign of accelerated decline in contrast sensitivity at either testing period. Thus, the deleterious Pb-aging interactions would appear to be dependent on the CNS function under study.

Recent studies in rats have demonstrated the importance of Pb exposure during early development in promoting the emergence of Alzheimer's-like changes in old age. Basha et al. (2005) exposed rat offspring to Pb through the dam during the lactational period and monitored gene expression of beta-amyloid precursor protein (APP). APP mRNA was induced in neonates, and exhibited a delayed overexpression 20 months after exposure was terminated. At this point, APP protein and its beta-amyloidogenic product were increased, and a rise in the activity of the transcription factor Sp1, one of the regulators of the *APP* gene, was also present. The changes induced by early exposure to Pb could not be reproduced by exposure to the metal during senescence. It was concluded that environmental influences occurring during brain development predetermined the expression and regulation of APP later in life. Subsequent work observed the same responses in monkeys who had been exposed to Pb as infants (Zawia and Basha, 2005), arguing for both an environmental trigger and a developmental origin of Alzheimer's disease.

Gender

Relatively few animal studies have attempted a clear demonstration of gender differences for toxic responses to chronic Pb exposure. For example, Donald et al. (1986) reported enhanced social investigatory behavior in exposed mice that differed in time course between males and

females. A subsequent report (Donald et al., 1987) showed Pb-induced nonsocial activity decreased in females, but increased in males and also caused shorter latencies to aggression in males. Also, Yang et al. (2003) found a gender difference in rats in memory retrieval, wherein higher doses of Pb affected memory in males than in females.

A significant functional impact of such gender differences has recently emerged. Cory-Slechta et al. (2004) evaluated the effects of interactions of chronic Pb exposure and maternal restraint stress on offspring. Corticosterone levels, neurotransmitter changes, and FI operant behavior were measured in animals exposed only during gestational and lactational periods, creating blood Pb levels of 30 to 40 µg/dL. These workers found that synergistic effects of Pb and stress were observed more frequently in female offspring, and that Pb with (in females) or without (in males) stress permanently elevated corticosterone levels. It was proposed that these findings uncovered a new mechanism by which exposure could enhance susceptibility to diseases. Virgolini et al. (2005) used continuous Pb exposure in the drinking water to males beginning at weaning, creating a maximal blood Pb of 27 µg/dL, in combination with variable intermittent stress challenges and found that Pb alone decreased basal plasma corticosterone levels and glucocorticoid receptor binding. Novelty stress in combination with exposure was found to modify FI behavior. These findings support the results of Cory-Slechta et al. (2004) in suggesting the potential for hypothalamic-pituitary-adrenal axis-mediated effects of Pb on CNS function.

Virgolini et al. (2006) extended this line of investigation by exposing rats to Pb prior to breeding and continuing throughout gestation and lactation. The exposure created maximal blood Pb levels of 33 to 43 µg/dL at termination of exposure at weaning. Maternal restraint stress was applied on gestational days 16 and 17 in some of the animals. Female offspring were then tested for responsiveness to various stressors (i.e., restraint, novelty, cold) as measured by FI operant performance. The combination of exposure and maternal stress produced more changes in responsiveness than either factor alone: operant behavior was altered following both restraint and cold, and the corticosterone response was modified by cold. It thusly appears that maternal Pb exposure can permanently alter stress responsivity and does so with a profile of effects that differ from those produced by either exposure alone or maternal stress alone. These studies are also among the first to make clear delineations of the effects of exposure across gender.

Developmental Period of Exposure

While early development is well-known to represent a period of particular sensitivity to the neurotoxic effects of Pb, several studies have made comparisons to the CNS effects produced by exposure encompassing later periods of development. There has been some consistency across these behavioral, neurophysiologic, and neurochemical observations.

As discussed in Section 5.3.5, Rice and Gilbert (1990a) employed a nonspatial discrimination reversal task in monkeys using form and colors as cues. The group continuously exposed to Pb from birth exhibited the most impairment on this task, whereas the group exposed continuously beginning at 300 days of age displayed impairment, but not as great as that of the preceding group. Another group exposed to Pb from birth but discontinued at 400 days of age did not exhibit an effect when tested as adults, suggesting that beginning Pb exposure after infancy results in altered performance, while exposure also during infancy exacerbates the effect. Rice (1990) later tested these same monkeys on a spatial discrimination reversal task and again found that the group continuously exposed from birth exhibited the most impairment.

Rice (1992a) utilized the same exposure protocols, with blood Pb levels of 20 to 35 µg/dL, and tested monkeys on a multi FI-FR schedule of reinforcement at two different ages. Few effects were seen during the first testing period at 3 years of age, but at 7 to 8 years of age increased response run rates and decreased interresponse times in FI responding were evident in all three exposed groups. These results indicated that exposure during infancy was not required for a Pb effect on this task, and that exposure only during infancy was sufficient to produce alterations.

Gilbert et al. (1999b) used a model of synaptic plasticity, i.e., LTP in the hippocampal dentate gyrus, to examine the effects of Pb exposure encompassing various developmental periods in intact animals with maximal blood Pb levels of 35 to 40 µg/d. Similar effects were seen in groups continuously exposed from birth or continuously exposed from PND 30: the magnitude of population spike LTP was diminished and the threshold for induction of the phenomenon was elevated. A group exposed only during the lactational period displayed a diminished magnitude of excitatory postsynaptic potential (EPSP) LTP also, but the threshold for EPSP slope LTP induction was higher only in the group continuously exposed from birth. Thus, exposure restricted to the lactational period was less disruptive to LTP in adult animals than exposure beginning around birth or weaning.

Lasley et al. (1999) examined stimulated release of glutamate and GABA in the hippocampus to evaluate the effects of developmental Pb exposure in intact animals with maximal blood Pb levels of 39 to 45 µg/dL. Similar decreases in Ca^{2+}-dependent depolarization-induced glutamate and GABA release were observed in groups continuously exposed from conception or continuously exposed from PND 35. The pattern of changes induced in the group continuously exposed from the beginning of gestation was similar to that observed previously in a group continuously exposed from birth (Lasley and Gilbert, 1996), indicating that gestational exposure did not further enhance the impact of Pb beginning at birth when exposure in both groups extended into adulthood. The changes found in the group continuously exposed beginning in the early postweaning period demonstrated that exposure during early development is not required to produce changes in glutamate and GABA release. Reductions in stimulated release were also observed in a group exposed only during the gestational and lactational periods, indicating that Pb limited to early development is also sufficient to produce deficits in evoked transmitter release.

Altmann et al. (1993) also examined synaptic plasticity and learning in rats chronically exposed during early development, producing maximal blood Pb levels of 14 to 16 µg/dL, but the results were in contrast to the others cited in this section. These workers found impaired LTP and AAL in groups continually exposed from the beginning of gestation, even if Pb was terminated in the early developmental period. Another group whose exposure began just prior to weaning did not display any Pb-related differences from controls.

These studies indicate that continuous exposure begun pre- or perinatally consistently results in a Pb effect as great as or greater than that produced by exposure over any other developmental period. Continuous exposure begun postweaning also is consistently potent in producing alterations, but whether the magnitude of the effect is similar to that of the preceding group, and whether exposure limited to the gestational and/or lactational period elicits an effect, appear to be task- or process-dependent.

Nutrition

It has long been known that diets sufficiently high in minerals such as zinc, iron, and calcium offer some protection from Pb exposure by competing with Pb^{2+} for absorption from the gastrointestinal tract. A full discussion of gastrointestinal absorption in humans is found in

Chapter 4, and information on absorption in animals is contained in Section 5.10.2. In fact, this dietary influence may comprise one component of the socioeconomic status (SES) factor repeatedly identified as important in studies of Pb neurotoxicity in children, as maternal dietary habits influence risk of exposure in infants. More recent studies have shown that vitamin D administration can reduce blood and bone Pb values (Cheng et al., 1998; Cortina-Ramirez et al., 2006), but whether this occurs as a result of increased Ca^{2+} uptake has not been determined. Diets designed to reduce caloric intake and increase weight loss have also been associated with increases in blood Pb values (Han et al., 1999).

Thresholds for CNS Effects

There is no evidence in the animal Pb neurotoxicity literature reflecting well-defined thresholds for any of the toxic mechanisms of the metal. Most studies performed with in vivo models report blood Pb values in the range of 15 to 35-40 µg/dL or higher. Moreover, in view of the complex and undefined speciation equilibria and distribution of Pb^{2+} in physiological milieus, there is no way to directly relate a blood Pb value to the levels of free Pb^{2+} ion or to any other complexed active form of the metal, either in extracellular or intracellular fluids. Generally accepted estimates of the free Pb^{2+} ion concentrations produced in brain extracellular fluid by environmentally relevant exposures fall in the low nanomolar range.

Nonetheless, changes induced by chronic Pb exposure have been reported on neurochemical (e.g., Cory-Slechta et al., 1997b) and neurophysiologic (e.g., Altmann et al., 1993) measures at blood Pb values of ~15 µg/dL. Recent studies have reported behavioral changes at blood Pb concentrations of ~10 µg/dL (Brockel and Cory-Slechta, 1998; 1999a,b), and the results on measures of attention have closely paralleled those observed in children at the same blood Pb levels. These observations serve to validate the accuracy and usefulness of the animal exposure models.

5.3.8 Summary

- Pb^{2+}-Ca^{2+} interactions resulting from exposure, e.g., the Ca^{2+}-mimetic properties of Pb^{2+}, are important components of the cellular toxicity of the metal and are closely related to the dose-dependent effects of exposure on neurotransmitter release. Some of these actions of Pb^{2+} are shown in Figure 5-3.

- Exposure-induced decreases in glutamatergic, cholinergic, and dopaminergic transmission are important because of the purported role of these neuronal systems in brain development and cognitive function.

- The majority of the data suggests an upregulation of NMDA receptors resulting from chronic exposure, but a consensus on the effects of Pb on NMDA receptor subunit expression and function remains to be attained. It is increasingly apparent that this glutamate receptor subtype may not be a direct primary target of chronic exposure in the intact animal.

- In vitro interactions of Pb^{2+} and PKC have been carefully described and are broadly relevant to cellular signaling pathways, but functional effects of these interactions in intact animals have not been achieved.

- Using hippocampal models of synaptic plasticity, it has been demonstrated that Pb exposure decreases the magnitude of LTP and increases the threshold for induction with a biphasic dose-effect relationship, another indication of the nonlinear effects of Pb.

- Decreases in stimulated glutamate release are a significant factor contributing to Pb-induced changes in LTP.

- Lead induces decreased activity of dopaminergic cells in substantia nigra and ventral tegmental area and inhibits activation of nicotinic currents in cultured hippocampal cells.

- Evidence from nonhuman primate studies demonstrates convincingly that Pb exposure producing blood Pb values as low as 33 µg/dL impairs auditory function by increasing latencies in brainstem auditory evoked potentials and elevating hearing thresholds.

- It has been shown that Pb exhibits selective effects on rod and bipolar cells in rats at a blood Pb of 19 µg/dL, causing decreases in maximal ERG amplitude, decreases in ERG sensitivity, and increases in mean ERG latency.

- Mechanisms for Pb-induced deficits in visual function include concentration-dependent inhibition of cGMP phosphodiesterase, increased rod Ca^{2+} levels, decreased retinal Na-K-ATPase activity, and apoptotic death of rod cells.

- Developmental exposure to Pb, creating steady state blood Pb levels of ~10 µg/dL, results in behavioral impairments that persist into adulthood in monkeys. There is no evidence of a threshold and Pb-induced deficits are, for the most part, irreversible.

- In monkeys, permanent neurobehavioral deficits are observed both with in utero-only exposure and with early postnatal-only exposure when peak blood Pb levels did not exceed 15 µg/dL and steady state levels were ~11µg/dL. Exposure started at ~300 of age creates deficits similar to those created with exposure from birth onward. In rats, permanent deficits are observed with prenatal, preweaning, and postweaning exposure.

- The developmental period of exposure is critical to the type of behavioral deficit produced.

- Lead affects the performance on a number of neurobehavioral tasks by inducing (1) reduced ability to inhibit inappropriate responding, (2) distractibility, (3) reduced ability to adapt to changes in behavioral contingencies and, (4) perseveration.

- Response perseveration, insensitivity to changes in reinforcement density or contingencies, and deficits in attention appear to be important mechanisms of Pb-induced learning deficits. Impulsivity and waiting-for-reward behavior may be more strongly affected by Pb than are sustained attention and hyperkinesis. Pb-induced impulsivity may be factor in cognitive impairments.

- Pb impairs learning at blood Pb levels as low as 11 µg/dL as tested in FI tasks. Performance on spatial and nonspatial discrimination reversal tasks is affected following developmental exposures in nonhuman primates. Distracting irrelevant stimuli potentiate these impairments. Pb also impairs performance on olfactory reversal tasks in rats. Discrimination reversal appears to be a more sensitive indicator of Pb-induced learning impairment than simple discrimination. Repeated-acquisition tests show that these deficits are unlikely to be caused by sensory or motor impairment.

- The effects of lead on memory are not as clear-cut: in some cases, the animals showed impairment of memory at blood Pb levels of as low as 10 µg/dL while in other studies animals demonstrated improved memory following Pb exposure. Short-term memory does not appear to be affected by low level Pb exposure. Some behavioral deficits in tests of working memory (e.g., DSA) appear to result from impaired attention rather than memory.

- Lead has been demonstrated to affect reactivity to environmental stimuli and social behavior in both rodents and nonhuman primates at blood Pb levels of 15 to 40 µg/dL.

- Other test paradigms such as drug discrimination and repeated acquisition/performance tasks have provided useful assessments of the integrity of CNS neurotransmitter systems in Pb exposed animals. Evidence from both methods has been in general agreement in indicating upregulated neurotransmitter receptor systems.

- Pb has been shown to decrease cell proliferation in vivo at 20 µg/dL and to decrease proliferation, differentiation, and neurite outgrowth in vitro.

- In both the adult and developing brain, the blood brain barrier (BBB) allows entry of Pb into the brain. Entry is dependent upon the chemical form of the Pb and interactions with proteins and other components of blood.

- The rate at which Pb accumulates in the brain depends upon the level and duration of exposure. The half-life of Pb in brain tissue is ~20 days in rats and appears to be homogenous across regions.

- Susceptibility and vulnerability factors that modify responses to lead include (1) age, (2) gender, (3) socioeconomic status, (4) period of exposure, and (5) nutrition.

- A well-defined threshold for any toxic effects in animals has not been identified. Neurochemical and neurophysiologic effects have been seen at blood Pb values of ~15 µg/dL and neurocognitive effects have been observed at ~10 µg/dL.

5.4 REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF LEAD

5.4.1 Summary of Key Findings on the Developmental and Reproductive Effects of Lead in Animals from the 1986 Lead AQCD

The 1986 Lead AQCD presented unequivocal evidence for effects of Pb on reproduction and development in laboratory animals, derived principally from studies of rodents. Fetotoxic effects (spontaneous abortion and fetal death) were reported following chronic exposures to relatively high doses (600 to 800 ppm) of inorganic Pb in the diet, and more subtle effects (such as changes in ALAD activity or hematocrit in offspring) at lower doses (5 to 10 ppm in drinking water and 10 $\mu g/m^3$ in air). The 1986 Lead AQCD reported that the lowest observed adverse effect level (LOAEL) for reproductive and developmental effects was 64 µg/kg per day (multiple exposures by gavage).

The 1986 Lead AQCD also reported evidence for a variety of sublethal effects on reproduction and development in experimental laboratory animals following Pb exposure. Sublethal effects included changes in levels or function of reproductive hormones as well as effects on the gonads (both male and female) and conception. The animal data also suggested more subtle effects on hormone metabolism and reproductive cell structure. Stowe and Goyer (1971) classified the reproductive effects of Pb as gametotoxic, whether intrauterine or extrauterine.

The data reported in the 1986 Lead AQCD, and more recent studies conducted in experimental animal models, provide convincing evidence that Pb induces temporary and long-lasting effects on male and female reproductive and developmental function. The newer literature supports the earlier conclusions presented in the 1986 Lead AQCD that Pb disrupts endocrine function at multiple points along the hypothalamic-pituitary-gonad axis (Sokol et al., 1985; Stowe and Goyer, 1971; Vermande-Van Eck and Meigs, 1960; Junaid et al., 1997; McGivern at al., 1991; Ronis et al., 1996, 1998b,c; Sokol, 1987; Sokol et al., 1985, 1994, 1998; Sokol and Berman, 1991; Kempinas at al., 1988, 1990, 1994; Tchernitchin et al., 1998b; Sant'

Ana et al., 2001; Srivastava et al., 2004). A schematic representation of the hypothalamic-pituitary-gonadal (HPG) axis is shown in Figure 5-7.

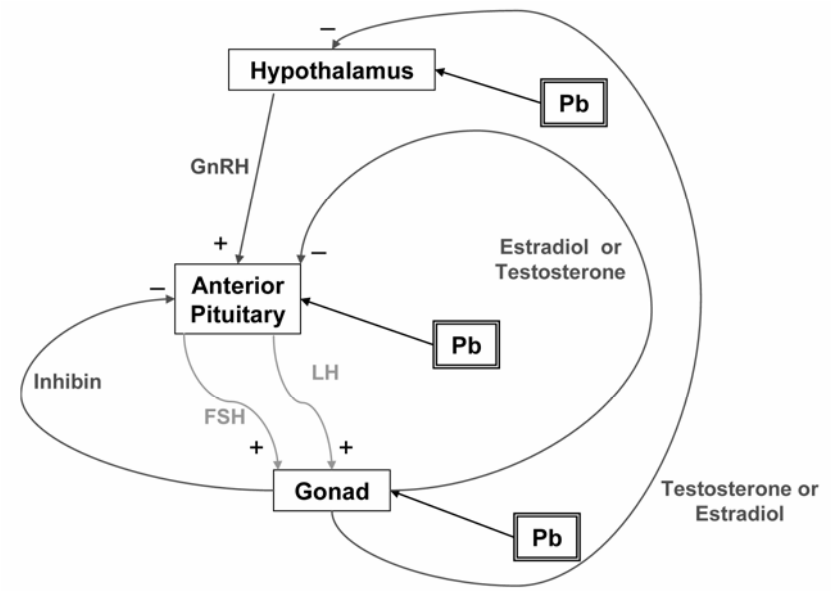

Figure 5-7. Data from male and female experimental animals suggests that lead has multiple targets in the hypothalmic-pituitary-gonadal axis.

The majority of the experimental animal studies on developmental and reproductive effects of Pb examined effects due to inorganic forms of lead; very little is known about the reproductive and developmental effects due to organic forms. In general, the few available studies suggest that effects of organic forms of Pb are similar to those produced by inorganic forms. Administration of triethyl-Pb-chloride during early gestation reduces pregnancy rates in mice (Odenbro and Kihlström, 1977). Growth retardation following organolead exposure has been reported (Kennedy et al., 1975; McClain and Becker, 1972). More recent studies have demonstrated that exposure of mice to triethyl-Pb-chloride during late gestation reduces perinatal growth rate (Odenbro et al., 1988).

This section summarizes the evidence for effects of Pb exposure in developing organisms exposed during the period from conception to maturity that has been reported since 1986. Effects on neurological, immunological, or renal endpoints in developing organisms are discussed in Sections 5.3, 5.9 and 5.7, respectively.

5.4.2 Effects on Male Reproductive Function

The 1986 Lead AQCD reported convincing evidence based on experimental animal studies that Pb acts as an endocrine disruptor in males. Those studies demonstrated an association between reduced male fertility and repeat-dose Pb exposure. Lead exposure had been reported to alter sperm development and function; however, the mechanism underlying these effects was not completely understood. These effects were attributed to either alterations in testicular enzymes important for hormone production or to changes in the hormone receptors. More recent research supports the conclusion that mechanisms for endocrine disruption in males involve lead acting at multiple sites along the hypothalamic-pituitary-gonadal axis (see Figure 5-7).

Reported effects of Pb on male reproduction differ substantially across studies, with some studies finding severe adverse effects and other studies finding no or minimal effects. The variable findings have been attributed to the complex mechanisms involved in hormone regulation and the multiple sites of action for Pb. Sokol et al. (2002) suggested that differences in results among studies may be, in part, attributed to an adaptive mechanism in the hypothalamic-pituitary-gonadal axis that may render the expression of some toxic effects dependent on dose and exposure duration. The mechanisms underlying this possible adaptation have not been completely elucidated. Lead exposure produces, a dose (blood Pb)-related suppression of serum testosterone levels and spermatogenesis, with an increase in GnRH mRNA in hypothalamus (at PbB <50). The latter effect is attenuated at higher exposures (>50 µg/dL) and with increasing exposure duration (Sokol et al., 2002). Sokol and Berman (1991) found that timing of exposure was critical to Pb-induced male reproductive toxicity in rats. Studies conducted in nonhuman primates supported the importance of timing, found that the adverse effects of Pb on male reproduction are dependent upon age (i.e., developmental stage at time of exposure) and duration of exposure (Foster et al., 1993; Singh et al., 1993a). It is currently unclear whether these effects reflect a physiological adaptation to the stress of lead exposure, or reflect the combined outcome of distinct dose-duration-response relationships for multiple effects on the HPG axis.

The adverse effects of Pb on male reproduction may be expressed as perturbations in sexual development and maturation, changes in fertility, endocrine disruption, and alterations in

structure of reproductive cells or tissue. Each of these effects is discussed in greater detail in the sections that follow.

5.4.2.1 Effects on Male Sexual Development and Maturation

The 1986 Lead AQCD reported adverse effects of Pb on male sexual development and maturation. Experimental studies conducted in animals demonstrated that high-dose (e.g., dietary exposure to 0.08 to 1.0% (800-1000 ppm) Pb acetate in mice and to 100 ppm in dogs) preadolescent Pb exposure can produce long-lasting detrimental effects on male sexual development. Numerous more recent studies conducted in experimental animals support the earlier findings that Pb exposure during early development can delay the onset of male puberty and alter reproductive function later in life (McGivern et al., 1991; Al-Hakkak et al., 1988; Chowdhuri et al., 2001; Dearth et al., 2002, 2004; Gandley et al., 1999; McGivern et al., 1991; Ronis et al., 1998a,c; Sokol et al., 1994; Yu et al., 1996). Studies that provide the strongest evidence for the dose-response range for typical effects in rodents are discussed below (Table 5-3).

McGivern et al. (1991) found that male rats born to dams that received Pb acetate in drinking water beginning on gestation day 14 and through parturition (blood Pb 73 µg/dL) exhibited reduced sperm counts, altered male reproductive behavior, and enlarged prostates later in life. Prepubertal exposure of male Sprague-Dawley rats (age 24 to 74 days) to Pb acetate in drinking water (blood Pb 30 to 60 µg/dL) resulted in significant reduction in testis weight and in the weight of secondary sex organs; however, these effects were not observed in rats exposed postpubertally (day 60 to 74; Ronis et al., 1996). A dose-dependent delay in sexual maturation was found in male rats, following prenatal Pb exposure that continued until adulthood (age 85 days) (Ronis et al., 1998a,b,c). In these studies, blood Pb levels in the pups between the ages of 21 and 85 days were >100 µg/dL. Additional details concerning these studies are provided in Table 5-3.

One possible explanation for the observed persistent effects of Pb exposure on the male reproductive system is a disruption in pulsatile release of sex hormones during early development (Ronis et al., 1998c). Lead effects on sex hormones are discussed in Section 5.4.2.3.

Table 5-3. Selected Studies Showing the Effects of Lead on Reproductive Function in Males

Citation	Species/ Strain	Dose/Route/Form/Duration/Group Size	Endpoint/Magnitude of Effect/p-value	Blood Lead Concentration (PbB)
Foster et al. (1993)	Monkey/ Cynomolgus	0–1500 µg Pb acetate/kg-d in gelatin capsules p.o. for various durations: 9 control monkeys, 4 monkeys in lifetime group (birth to 9 years), 4 in infancy group (first 400 days of life), 4 in post-infancy exposure (from 300 days to 9 years)	Suppressed LH response to GnRH stimulation in the lifetime group ($p = 0.0370$); Sertoli cell function (reduction in the inhibin to FSH ratio) ($p = 0.0286$) in lifetime and post-infancy groups.	Lifetime group 3–26 µg/dL at 4–5 years Infancy group 5–36 µg/dL at 100–300 days, 3–3 µg/dL at 4–5 years Post-infancy group 20–35 µg/dL
Foster et al. (1996a)	Monkey/ Cynomolgus	0–1500 µg Pb acetate/kg-d in gelatin capsules p.o. from birth until 9 years of age 8 control monkeys, 4 monkeys in low group (6–20 µg/dL), 7 monkeys in high group (22–148 µg/dL)	Mean PbB of 56 µg/dL showed no significant alterations in parameters of semen quality (count, viability, motility, or morphology).	PbB 10 ± 3 or 56 ± 49 µg/dL
Foster et al. (1998)	Monkey/ Cynomolgus	0–1500 µg Pb acetate/kg-d in gelatin capsules p.o. for various durations: birth to 10 years (lifetime); PND 300 to 10 years (post-infancy); birth to 300 days (infancy); 3 control monkeys, 4 lifetime, 4 infancy, 5 post-infancy	Circulating concentrations of FSH, LH, and testosterone were not altered by treatment; semen characteristics (count, motility, morphology) were not affected by treatment possibly because not all Sertoli cells were injured; degeneration of seminiferous epithelium in infancy and lifetime groups (no difference in severity between these groups); ultrastructural alterations in seminal vesicles, most prominent in infancy and post-infancy groups.	PbB ~35 µg/dL
McGivern et al. (1991)	Rat/Sprague-Dawley	0.1% Pb acetate in drinking water from GD 14 to parturition; 8 control litters; 6 Pb acetate litters (5 males per litter)	Decreased sperm count (21% at 70 days and 24% at 165 days, $p < 0.05$); reduced male behavior ($p < 0.05$); enlarged prostate (25% increase in weight; $p < 0.07$); irregular release patterns of both FSH and LH ($p < 0.05$).	Control PbB <5 µg/dL at birth Maternal PbB 73 µg/dL at birth Pup PbB 64 µg/dL at birth
Ronis et al. (1996)	Rat/Sprague-Dawley	0.6% Pb acetate in drinking water for various durations: PND 24–74 (pubertal exposure); PND 60–74 (post pubertal exposure); 11 males and females in pubertal exposure group (10 each in control pubertal group); 6 males and females post-pubertal exposure and control groups	PbB >250 µg/dL reduced circulating testosterone levels in male rats 40–50% ($p < 0.05$); reduction in male secondary sex organ weight ($p < 0.005$); delayed vaginal opening ($p < 0.0001$); disrupted estrous cycle in females (50% of rats); increased incidence of stillbirth (2% control vs. 19% Pb) ($p < 0.005$).	Pubertal PbB 30–60 µg/dL Post-pubertal PbB 30–60 µg/dL Mean PbBs in male rats 30–60 µg/dL, respectively

Table 5-3 (cont'd). Selected Studies Showing the Effects of Lead on Reproductive Function in Males

Citation	Species/ Strain	Dose/Route/Form/Duration/Group Size	Endpoint/Magnitude of Effect/p-value	Blood Lead Concentration (PbB)	
Ronis et al. (1998a)	Rat/Sprague-Dawley	0.6% Pb acetate in drinking water ad libitum for various durations as follows: GD 5 to PND 1; GD 5 to weaning; PND 1 to weaning; 3 control litters, 2 gestation exposure litters, 2 lactation exposure litters, 2 gestation and lactation exposure litters, 2 postnatal exposure litters, 2 chronic exposure litters; 4 male and 4 female pups per litter	Suppression of adult mean serum testosterone levels was only observed in male pups exposed to Pb continuously from GD 5 throughout life ($p < 0.05$).	Group	Male PbB
				Naïve	5.5 ± 2.0 µg/dL
				Control	1.9 ± 0.2 µg/dL
				Gest	9.1 ± 0.7 µg/dL
				Lact	3.3 ± 0.4 µg/dL
				Gest+Lact	16.1 ± 2.3 µg/dL
				Postnatal	226.0 ± 29 µg/dL
				Chronic	316.0 ± 53 µg/dL
Ronis et al. (1998b)	Rat/Sprague-Dawley	Lead acetate in drinking water (0.05% to 0.45% w/v); dams exposed until weaning; exposure of pups which continued until PND 21, 35, 55, or 85; 5 control litters (0%), 10 low-dose litters (0.05%), 8 mid-dose litters (0.15%), 9 high-dose litters (0.45%); 4 male and 4 female pups per litter	Dose-response reduction in birth weight ($p < 0.05$), more pronounced in male pups; decreased growth rates in both sexes ($p < 0.05$) were accompanied by a statistically significant decrease in plasma concentrations of IGF1 through puberty PND 35 and 55 ($p < 0.05$); increase in pituitary growth hormone during puberty ($p < 0.05$).	Mean PbB in offspring at 0.05% (w/v) 49 ± 6 µg/dL Mean PbB in offspring at 0.15% (w/v) 126 ± 16 µg/dL Mean PbB in offspring at 0.45% (w/v) 263 ± 28 µg/dL	
Ronis et al. (1998c)	Rat/Sprague-Dawley	Lead acetate 0.05, 0.15, or 0.45% in drinking water beginning GD 5 continuing until PND 21, 35, 55, or 85; 5 control litters (0%), 10 low-dose litters (0.05%), 8 mid-dose litters (0.15%), 9 high-dose litters (0.45%); 4 male and 4 female pups per litter	Dose-responsive decrease in birth weight ($p < 0.05$); dose-responsive decrease in crown-to-rump length ($p < 0.05$); dose-dependent delay in sexual maturity ($p < 0.05$); decrease in prostate weight ($p < 0.05$); decrease in plasma concentration of testosterone during puberty ($p < 0.05$); decrease in plasma LH ($p < 0.05$); elevated pituitary LH content ($p < 0.05$); decrease in plasma testosterone/LH ratio at high dose ($p < 0.05$).	Dams: 0, 48, 88, or 181 µg/dL Pups PND 1: <1, 40, 83, or 120 µg/dL Pups PND 21: <1, 46, 196, or 236 µg/dL Pups PND 35: <1, 20, 70, or 278 µg/dL Pups PND 55: <1, 68, 137, or 379 µg/dL Pups PND 85: <1, 59, 129, or 214 µg/dL	

Table 5-3 (cont'd). Selected Studies Showing the Effects of Lead on Reproductive Function in Males

Citation	Species/ Strain	Dose/Route/Form/Duration/Group Size	Endpoint/Magnitude of Effect/p-value	Blood Lead Concentration (PbB)		
Singh et al. (1993a)	Monkey/ Cynomolgus	0–1500 µg Pb acetate/kg-d in gelatin capsules for various durations: 3 control monkeys, 4 monkeys in infancy group (exposure first 400 days), 5 in post-infancy group (exposure 300 days to 9 years of age), 4 in lifetime group (exposure from birth until 9 years)	Degeneration of seminiferous epithelium in all exposed groups (frequency not specified); ultrastructural alterations in seminal vesicles, most prominent in infancy and post-infancy groups (frequency not specified).	Chronic PbB <40–50 µg/dL		
Sokol and Berman (1991)	Rat/Wistar	0, 0.1, or 0.3% Pb acetate in drinking water for 30 days beginning at 42, 52, or 70 days old; 8–11 control rats for each age, 8–11 rats for each age in 0.1% group, 8–11 rats for each age in 0.3% group	Dose-related suppression of spermatogenesis (decreased sperm count and sperm production rate) in the exposed rats of the two highest age groups ($p < 0.05$); dose-related suppression of serum testosterone in 52-day old rats ($p = 0.04$) and in 70-day old rats ($p < 0.003$).	Group	Age	PbB
				0%	All	<7 µg/dL
				0.1%	42 d	25 µg/dL
					52 d	35 µg/dL
					70 d	37 µg/dL
				0.3%	42 d	36 µg/dL
					52 d	60 µg/dL
					70 d	42 µg/dL

FSH, follicle stimulating hormone; GD, gestational day; GnRH, gonadotropin releasing hormone; IGF_1, insulin-like growth factor 1; LH, luteinizing hormone; PbB, blood Pb concentration; PND, post-natal day

5.4.2.2 Effects on Male Fertility: Effects on Sperm Production and Function

The 1986 Lead AQCD presented evidence that Pb exposure affects male fertility in various animal species, including rabbits (Cole and Bachhuber, 1915), guinea pigs (Weller, 1915), rats (Ivanova-Chemishanska et al., 1980), and mice (Schroeder and Mitchener, 1971).

Several more recent studies, conducted in various animal species, have demonstrated Pb-induced alterations of sperm parameters (e.g., count, motility, number of abnormal) (Sokol et al., 1985; and eight other studies). These effects, however, have not been reproduced in all studies. For example, Foster et al. (1996a) reported that 15- to 20-year-old cynomolgus monkeys receiving Pb acetate for their lifetime (mean blood Pb 56 µg/dL) showed no significant alterations in sperm parameters (i.e., sperm count, viability, motility, and morphology) or circulating levels of testosterone (see Section 5.4.2.3 for discussion of Pb-induced changes in testosterone levels). Adaptive (Sokol et al., 2002) or multiple effects on the HPG axis having different dose-duration-response relationships may explain the apparent inconsistency in reported effects on circulating testosterone levels, sperm count, and sperm production following Pb exposure. As a result, changes in testosterone levels and certain sperm parameters may not always serve as reliable endpoints for assessing the effects of Pb on male fertility and reproductive function for all exposure durations.

Although gross changes in sperm parameters were not observed in monkeys in which chronic blood Pb was ~56 µg/dL, Foster et al. (1996a) reported that monkey sperm exhibited a statistically significant, dose-related reduction in chromatin structure (as determined by susceptibility to weak acid denaturation). These changes may have adverse impacts on fertility, and they are thought to be related to dominant lethal effects of Pb (similar to the effects reported by al-Hakkak et al. [1988] in mice). Additional details concerning Foster et al. (1996a) are provided in Table 5-3.

The data from Foster et al. (1996a), demonstrating a change in monkey sperm chromatin suggestive of a subtle lead-induced reduction in male fertility (in the absence of gross changes in sperm parameters), are consistent with observations of reduced in vitro fertilization capacity of sperm collected from other mammalian species. Sokol et al. (1994) reported that exposure of adult male rats to Pb acetate in drinking water for 14 to 60 days (blood Pb 33 to 46 µg/dL) resulted in reduced in vitro fertilization of eggs harvested from unexposed females. No differences were observed in sperm ultrastructure or in the DNA histogram of sperm obtained

from Pb-exposed rats compared to controls. Consistent with this finding are reports of reduced fertilization capacity of rabbit sperm exposed to high concentrations (25 μM) of Pb chloride in vitro (Foote, 1999) and reduced in vitro fertilization capacity of sperm from mice exposed to Pb in drinking water at 1 g/L for 4 months (blood Pb concentration not reported) (Johansson et al., 1987).

Two modes of action have been proposed for Pb-induced alterations in sperm capacity for fertilization. The affinity of Pb for sulfhydryl groups may explain some of the Pb-induced alterations in sperm structure and function. Mammalian sperm possess high concentrations of sulfhydryl groups, including chromatin stabilizing protamines, which are critical for maintenance of normal function (Johansson and Pellicciari, 1988; Quintanilla-Vega et al., 2000). Reyes et al. (1976) demonstrated that binding of Pb to membrane thiols inhibits sperm maturation. In addition, recent experimental data also suggest that Pb-induced generation of reactive oxygen species (ROS) may contribute to the injury of tissues responsible for sperm formation (see Section 5.4.2.4).

5.4.2.3 Effects on Male Sex Endocrine System

The 1986 Lead AQCD reported that, although the mode of action for the adverse effects of Pb on the male reproductive system was not understood, effects on hormone production or hormone receptors were likely contributors. More recent studies provide convincing evidence that Pb acts as an endocrine disruptor in males at various points along the hypothalamic-pituitary-gonadal axis (Figure 5-7). In rats, Pb exposures that decreased serum testosterone levels increased mRNA levels of GnRH and LH in the hypothalamus and pituitary, respectively, and increased levels of LH in pituitary; these changes can occur in the absence of a change in serum gonadotropin levels (Klein et al., 1994; Ronis et al., 1998c; Sokol et al., 2002). In monkeys, chronic Pb exposures (blood Pb 20 to 35 μg/dL) suppressed GnRH-induced secretion of LH and decreased serum testosterone:LH and inhibin:FSH ratios (Foster et al., 1993). The mechanisms underlying the effects on the hypothalamic-pituitary-gonadal axis have not been elucidated but may involve a suppression of GnRH secretion (Bratton et al., 1994; Sokol, 1987; Sokol et al., 1998).

Although there is evidence for a common mode of action, consistent effects on circulating testosterone levels are not always observed in Pb-exposed animals. Rodamilans et al. (1988) and

Kempinas et al. (1994) attributed these inconsistencies to the normal biological variation (circannual and seasonal) of testosterone secretion in rats and monkeys. Observations of Pb-induced reductions in testosterone levels in some studies, but not others, may be due to enhanced sensitivity to inhibition of the testosterone secretory system during certain periods of development. In addition, compensatory mechanisms in the hypothalamic-pituitary-gonad axis may attenuate some effects of Pb during prolonged Pb exposure (Sokol et al., 2002). Taken together, the sensitivity of testosterone secretion during certain periods and potential for modulation of the effects during long-term exposures studies, may explain some of the apparent inconsistencies in the reported effects of Pb exposure on circulating testosterone levels.

5.4.2.4 Effects on Morphology and Histology of Male Sex Organs

The 1986 Lead AQCD reported evidence for histological changes in the testes or prostate in rats, in association with relatively high doses of Pb (Chowdhury et al., 1984; Hilderbrand et al., 1973; Golubovich et al., 1968). More recent studies conducted in animal models provide persuasive support for testicular damage, i.e., ultrastructural changes in testes and cytotoxicity in Sertoli cells (Foster et al., 1998; Singh et al., 1993a; Batra et al., 2001; Chowdhury et al., 1986, 1987; Corpas et al., 1995; Pinon-Lataillade et al., 1993; Saxena et al., 1990). Studies conducted in nonhuman primates warrant particular attention. These studies found ultrastructural changes in the testes (Sertoli and other spermatogenic cells) of monkeys at blood Pb 35 to 40 µg/dL (Foster et al., 1998; Singh et al., 1993a).

Foster et al. (1998) reported that chronic Pb exposure (blood Pb ~35 µg/dL), beginning in infancy, resulted in persistent ultrastructural changes in the testes of cynomolgus monkeys. Electron microscopy showed disruption of the general structure of the seminiferous epithelium involving Sertoli cells, basal lamina, and spermatids in the groups exposed for lifetime and during infancy only (no duration difference in severity). Chronic exposures to Pb beginning after infancy, that achieved similar blood Pb levels, did not produce these effects.

Similarly, Singh et al. (1993a) demonstrated ultrastructural changes in testicular basement membrane and Sertoli cell morphology (seminiferous tubules) in cynomolgus monkeys exposed chronically to Pb (blood Pb <40 to 50 µg/dL); the effects were most prominent when dosing began in infancy or post-infancy. These results suggest that, in monkeys, Pb exposure during

certain periods of development produces persistent testicular alterations. Additional details concerning Foster et al. (1998) and Singh et al. (1993a) are provided in Table 5-3.

A possible mode of action for Pb-induced testicular injury is oxidative stress. Foster et al. (1998) suggested that Pb-induced oxygen free radical generation was a plausible mechanism of testicular injury in primates. This oxygen radical hypothesis is supported by studies conducted in rodents (Chowdhury et al., 1984; Acharya et al., 2003; Adhikari et al., 2001; Batra et al., 2001; Bizarro et al., 2003; Chowdhury et al., 1984; Gorbel et al., 2002; Mishra and Acharya, 2004). Also supporting the oxidative stress hypothesis are observations of increases in the percentage of apoptotic cells in the testes of rodents in response to Pb exposure (Pace et al., 2005; Gorbel et al., 2002; Adhikari et al., 2001).

Studies in experimental animals (assessed in the 1986 Lead AQCD and others published subsequent to the 1986 Lead AQCD) provide convincing evidence that Pb acts as an endocrine disruptor in males. The majority of present studies support the conclusion that endocrine disruption in males involves Pb acting at multiple sites along the hypothalamic-pituitary-gonadal axis. The adverse effects of Pb on male reproduction include perturbations in sexual development and maturation, changes in fertility, changes in male sex hormone levels, and alterations in gonad tissues and cell structure.

5.4.3 Effects on Female Reproductive Function

Lead has been shown to disrupt the hypothalamic-pituitary-gonadal axis and to produce ovarian atrophy and reproductive dysfunction in females (Figure 5-7). The 1986 Lead AQCD reported that Pb exposure was associated with inhibition of menstruation, ovulation, and follicular growth in monkeys (Vermande-Van Eck and Meigs, 1960), and, in rodents, Pb exposure delayed vaginal opening, decreased frequency of implantation, and reduced rates of pregnancy (Kimmel et al., 1980; Odenbro and Kihlström, 1977, respectively).

Data from more recent experimental animal studies support these findings. Lead effects on female reproduction may be classified as alterations in female sexual maturation, effects on fertility and menstrual cycle, endocrine disruption, and changes in morphology or histology or female reproductive organs as well as the placenta. Recent literature concerning each of these effects is summarized below.

5.4.3.1 Effects on Female Sexual Development and Maturation

The 1986 Lead AQCD reported that Pb exposure in rodents produced delays in sexual maturation. Grant et al. (1980) reported delayed vaginal opening in female rats exposed in utero and during lactation and maturation (blood Pb ~20 to 40 µg/dL). More recent studies in experimental animals (primarily rodent studies) provide convincing evidence that Pb exposure before puberty (particularly prenatal and early postnatal exposure) delays the maturation of the female reproductive system (Dearth et al., 2002, 2004; Ronis et al., 1996, 1998b,c).

The study of Dearth et al. (2002) is of particular interest because it employed a cross-fostering design (to allow comparison of pups exposed during gestation only, lactation only, or both) and because maternal and offspring blood Pb were monitored throughout gestation and lactation. Fisher 344 dams were exposed to Pb by gavage beginning 30 days before mating until weaning of the pups at 21 days of age (gavage exposure removes possible confounding of exposure by consumption of Pb in drinking water by pups in those studies where drinking water is the route of exposure for dams). Mean maternal blood Pb level was ~40 µg/dL. Pups exposed during gestation and lactation had the highest blood Pb (38.5 µg/dL) on day 10; at this time, the blood Pb levels in pups exposed during gestation only or lactation only were 13.7 and 27.6 µg/dL, respectively. By postnatal day (PND) 30, all three groups had blood Pb ≤3 µg/dL. Dearth et al. (2002) reported a statistically significant delay in the onset of puberty (vaginal opening and days at first diestrus) in rats exposed during lactation, gestation, or during lactation and gestation (with no differences among the groups). In addition, statistically significant reductions in the circulating levels of insulin-like growth factor 1 (IGF_1), LH, and estradiol (E_2) were reported on PND 30 in all three treatment groups (with no differences among treatment groups). Additional details concerning Dearth et al. (2002) are provided in Table 5-4.

A subsequent study in both Sprague-Dawley and F344 rats (Dearth et al., 2004) showed that the F344 strain is more sensitive to maternal Pb exposure than Sprague-Dawley rats to Pb-induced delayed puberty, which could, in part, explain the inconsistencies with effect levels observed in Sprague Dawley rats (e.g., Ronis et al., 1998a,b,c; McGivern et al., 1991). Ronis et al. (1998c) suggested that the delayed onset of puberty may arise from a Pb-induced disruption of pulsatile release of sex hormones (see Section 5.4.3.3).

Table 5-4. Selected Studies Showing the Effects of Lead on Reproductive Function in Females

Citation	Species/ Strain	Dose/Route/Form/Duration/ Group Size	Endpoint/Magnitude of Effect (% or incidence)/p-value	Blood Lead Concentration (PbB)
Dearth et al. (2002)	Rat/Fisher 344	12 mg/mL Pb acetate gavage from 30 days prior breeding until pups were weaned 21 day after birth; 10–32 litters per group, control group, gestation and lactation exposure, gestation only exposure, lactation only exposure	Delay in onset of puberty ($p < 0.05$); reduced serum levels of IGF_1 ($p < 0.001$), LH ($p < 0.001$), and E_2 ($p < 0.001$).	Maternal PbB ~40 µg/dL Pups PbB as follows: Gest+lact ~38 µg/dL PND 10 Gest+lact ~15 µg/dL PND 21 Gest+lact ~3 µg/dL PND 30 Gest ~14 µg/dL PND 10 Gest ~3 µg/dL PND 21 Gest ~1 µg/dL PND 30 Lact ~28 µg/dL PND 10 Lact ~15 µg/dL PND 21 Lact ~3 µg/dL PND 30
Foster (1992)	Monkey/ Cynomolgus	Daily dosing for up to 10 years with gelatin capsules containing Pb acetate (1.5 mg/kg); 8 control group monkeys, 8 lifetime exposure (birth–10 years), 8 childhood exposure (birth–400 days), and 8 adolescent exposure (PND 300-10 years of age)	Statistically significant reductions in circulating levels of LH, ($p < 0.042$), FSH ($p < 0.041$), and E_2 ($p < 0.0001$) during menstrual cycle; progesterone concentrations were unchanged and menstrual cycle was not significantly affected.	PbB <40 µg/dL
Foster et al. (1992)	Monkey/ Cynomolgus	Daily dosing for up to 10 years with gelatin capsules containing Pb acetate (1.5 mg/kg); 8 control group monkeys, 8 childhood (birth–400 days), 7 adolescent (PND 300–10 years), 7 lifetime (birth–10 years)	No effect on endometrial response to gonadal steroids as determined by ultrasound.	PbB <40 µg/dL
Foster et al. (1996b)	Monkey/ Cynomolgus	Chronic exposure to Pb acetate 50 to 2000 µg/kg-day p.o. beginning at birth for 15–20 years; 20 control monkeys, 4 monkeys in 50 µg/kg-d group, 3 monkeys in 100 µg/kg-d, 2 monkeys in 500 µg/kg-d group, and 3 monkeys in 2000 µg/kg-d group	Reduced corpora luteal production of progesterone ($p = 0.04$), without alterations in E_2, 20-alpha-hydroxyprogesterone, or menstrual cyclicity.	PbB 10–15 µg/dL in low group (50 or 100 µg/kg-day) PbB 25–30 µg/dL in moderate group (500 or 2000 µg/kg-day)

Table 5-4 (cont'd). Selected Studies Showing the Effects of Lead on Reproductive Function in Females

Citation	Species/ Strain	Dose/Route/Form/Duration/ Group Size	Endpoint/Magnitude of Effect (% or incidence)/p-value	Blood Lead Concentration (PbB)
Franks et al. (1989)	Monkey/ Rhesus	Lead acetate in drinking water (2–8 mg/kg-d) for 33 months; 7 control and 10 Pb monkeys	Reduced circulating concentration of progesterone ($p < 0.05$); treatment with Pb did not prevent ovulation, but produced longer and more variable menstrual cycles and shorter menstrual flow.	PbB 68.9 ± 6.54 µg/dL
Laughlin et al. (1987)	Monkey/ Rhesus	Lead acetate in drinking water at 3.6, 5.9, or 8.1 mg/kg-day for 1–2 years 7 control and 10 experimental monkeys per group	Reductions in cycle frequency ($p < 0.01$); fewer days of flow ($p < 0.01$); longer and more variable cycle intervals ($p < 0.025$).	PbB 44–89 µg/dL 51.2 µg/dL (low dose) 80.7 µg/dL (mid dose) 88.4 µg/dL (high dose)
Lögdberg et al. (1988)	Monkey/ Squirrel	Lead acetate (varying concentrations ≤0.1% in diet) maternal dosing from 5-8.5 weeks pregnant to PND 1 11 control monkeys, 3 low Pb exposure group (PbB 24 µg/dL), 7 medium Pb group (PbB 40 µg/dL, 5 high Pb group (PbB 56 µg/dL)	Dose-dependent reduction in placental weight ($p < 0.0007$); various pathological lesions were seen in the placentas ($n = 4$), including hemorrhages, hyalinization of the parenchyma with destruction of the villi and massive vacuolization of chorion epithelium.	Mean maternal PbB 37 µg/dL (22-82 µg/dL) 24 (22–26) µg/dL (low dose) 40 (35–46) µg/dL (mid dose) 56 (43–82) µg/dL (high dose)

E_2, estradiol; FSH, follicle stimulating hormone; GD, gestational day; IGF_1, insulin-like growth factor 1; LH, luteinizing hormone; PbB, blood Pb concentration; PND, post-natal day

5.4.3.2 Effects on Female Fertility

The 1986 Lead AQCD reported convincing evidence from experimental animal studies for Pb-induced alterations in female fertility, including interference with implantation and pregnancy (Odenbro and Kihlström, 1977; Wide and Nilsson, 1977). More recent studies have confirmed these effects. In general, Pb exposure does not produce total sterility, although Pb exposure clearly disturbs female fertility (Taupeau et al., 2001). Studies in nonhuman primates and rodents have shown that exposure of gravid females to Pb produces implantation dysfunction and reduces litter size and newborn survival (Lögdberg et al., 1987; Flora and Tandon, 1987; Johansson and Wide, 1986; Pinon-Lataillade et al., 1995; Piasek and Kostial, 1991; Ronis et al., 1996). See Section 5.4.4.1 for details.

5.4.3.3 Effects on the Female Sex Endocrine System and Menstrual Cycle

The 1986 Lead AQCD described numerous studies that found effects of Pb on the female endocrine system and menstrual cycle in various species, including nonhuman primates, and that supported the conclusion that Pb was an endocrine disruptor in females (Grant et al., 1980; Maker et al., 1975; Vermande-Van Eck and Meigs, 1960). Observations of delayed vaginal opening (see Section 5.4.3.1) were attributed to the endocrine disruption effects of Pb on the hypothalamic-pituitary-gonadal axis (Stowe and Goyer, 1971; Vermande Van Eck and Meigs, 1960).

More recent studies have provided convincing support for endocrine-mediated alterations of the female reproductive system in rats (Srivastava et al., 2004; Dearth et al., 2002; Ronis et al., 1998a,b,c; Junaid et al., 1997; Ronis et al., 1996), guinea pigs (Sierra and Tiffany-Castiglioni, 1992), and nonhuman primates (Foster et al., 1992, 1996b; Foster, 1992; Franks et al., 1989; Laughlin et al., 1987). The nonhuman primate studies are particularly relevant to extrapolations to humans and provide dose-response information for effects of Pb on female sex hormones and menstrual cycle.

Laughlin et al. (1987) found that Pb exposure (blood Pb 44 to 89 µg/dL) alters menstrual cycles (specifically, causing reductions in cycle frequency, fewer days of menstrual flow, and longer and more variable cycle intervals) in female rhesus monkeys. Consistent with these observations, Franks et al. (1989) found that chronic exposure to Pb in the drinking water (blood Pb 70 µg/dL) reduced circulating concentrations of progesterone (suggesting impaired luteal

function), produced longer and more variable menstrual cycles and temporally shorter menstrual flow in female rhesus monkeys. Additional details concerning these studies are provided in Table 5-4.

At lower blood Pb levels (<40 µg/dL), female cynomolgus monkeys exhibited statistically significant reductions in circulating levels of LH, FSH, and E_2 during the menstrual cycle; however, serum progesterone concentrations were unchanged and menstrual cycle was not significantly affected (Foster, 1992). Similar exposures and blood Pb levels were shown to have no effect on endometrial response to gonadal steroids in cynomolgus monkeys as determined by ultrasound analysis (Foster et al., 1992). At lower blood Pb concentrations (25 to 30 µg/dL), reduced corpora luteal production of progesterone occurred in the absence of alterations in E_2, 20-alpha-hydroxyprogesterone, or menstrual cyclicity (Foster et al., 1996b). In contrast to Foster et al. (1992), this study (Foster et al., 1996b) found no statistically significant effect of Pb on serum progesterone levels in cynomolgus monkeys that had lower blood Pb levels (10 to 15 µg/dL). Additional details concerning these studies are provided in Table 5-4.

Several modes of action for Pb-induced, endocrine disruption-mediated alterations in female reproduction have been proposed, including changes in hormone synthesis or metabolism at the enzyme level (Wiebe and Barr, 1988; Wiebe et al., 1988) and changes in hormone receptor levels (Wiebe et al., 1988; Wide and D'Argy, 1986). In addition, Pb may alter sex hormone release and imprinting during early development (Ronis et al., 1998c; Tchernitchin et al., 1998a,b). The latter effects would be consistent with observations of persistent changes in estrogen receptor levels in the uterus (Wiebe and Barr, 1988) and LH function in the ovary (Srivastava et al., 2004) in Pb-exposed animals.

5.4.3.4 Effects on Morphology and Histology of Female Sex Organs and the Placenta

Lead-induced changes in morphology or histology in female sex organs and the placenta may explain reduced fertility and impaired female reproductive success (see Sections 5.4.3.2 and 5.4.4.1.). Lögdberg et al. (1988) reported a dose-dependent reduction in placental weight and an increase in pathological lesions of the placenta in squirrel monkeys that received oral doses of Pb acetate (0.001 to 0.1% in diet) during the last three-fourths or two-thirds of pregnancy (mean maternal blood Pb 37 µg/dL; range: 22 to 82 µg/dL). These effects occurred without overt

toxicity in the mothers. Additional details concerning Lögdberg et al. (1988) are provided in Table 5-4.

Similar effects on placental weight and histology were observed in mice (Fuentes et al., 1996; Nayak et al., 1989a). These effects on the placenta may explain the reduced birth weight that has been associated with prenatal Pb exposure (see Section 5.4.5). Exposure to Pb in early pregnancy also produces structural changes in the epithelium of the uterus of mice (Nilsson et al., 1991; Wide and Nilsson, 1979). These changes in uterine tissue may impair successful implantation of the blastocysts (see Section 5.4.4.1).

The 1986 Lead AQCD reported that Pb exposure (blood Pb 20 to 40 µg/dL) in rodents produced delays in sexual maturation. More recent studies in experimental animals (primarily rodent studies) provide convincing evidence that Pb exposure before puberty (prenatal and early postnatal blood Pb ~40 µg/dL) delays maturation of the female reproductive system (Dearth et al., 2002, 2004; Iavicoli et al., 2004; McGivern et al., 1991; Ronis et al., 1998a,b,c,). Ronis et al. (1998c) suggested that lead-induced disruption of pulsatile release of sex hormones may result in delayed onset of puberty.

5.4.4 Effects on Embryogenesis

Lead exposure can increase fetal mortality, produce a variety of sublethal effects, and disrupt the growth and development of the offspring. Many of the lead-induced sublethal developmental effects occur at maternal blood Pb levels that do not result in clinical toxicity in the mothers.

5.4.4.1 Embryo/Fetal Mortality

The 1986 Lead AQCD concluded that that acute exposure to high doses of Pb interfered with implantation and pregnancy (Wide, 1985; Odenbro and Kihlström, 1977; Wide and Nilsson, 1977; Vermande-Van Eck and Meigs, 1960). This conclusion is supported by results of more recent studies (Lögdberg et al., 1987; Giavini et al., 1980; Jacquet, 1976, 1977; Jacquet et al., 1975, 1976; Johansson and Wide 1986; Johansson et al., 1987; Johansson, 1989; Maisin et al., 1978; Pinon-Lataillade et al., 1995; Wide and Nilsson, 1977, 1979).

Lögdberg et al. (1987) reported an increase in pre- and perinatal mortality in squirrel monkeys that received Pb acetate orally during the last two-thirds of pregnancy (45% versus

7 to 8% among controls). The mean maternal blood Pb level was 54 μg/dL (39 to 82 μg/dL). These fetotoxic effects occurred without overt toxicity in the mothers. Additional details concerning Lögdberg et al. (1987) are provided in Table 5-5. These effects are consistent with data from rodent studies, wherein gestational exposure to Pb (blood Pb 32 to >70 μg/dL) resulted in smaller litters and fewer implantation sites (e.g., Pinon-Lataillade et al., 1995; Singh et al., 1993b; Piasek and Kostial, 1991). Numerous studies have been performed to elucidate possible mechanisms by which Pb causes prenatal death (Maisin at al., 1978; Jacquet, 1977, 1976; Jacquet et al., 1976, 1975). The available data suggest that Pb may alter blastocyst development and impair implantation. Hanna et al. (1997) demonstrated that in vitro exposure of 2- and 4-cell mouse embryos to 200 μM Pb acetate resulted in reduced cell proliferation and blastocyst formation. Additional evidence for an effect on blastocysts is provided by data from in vitro fertilization studies (Chowdhuri et al., 2001; Johansson, 1989; Johansson et al., 1987). Johansson and co-workers (1989, 1987) reported that Pb delayed the timing of escape of spermatozoa from the zona pellucida and induced a premature acrosome reaction. These effects could disrupt attachment and implantation of the blastocyst if they were to occur in vivo.

Observations from more recent experimental animal studies support these findings. The effects of Pb on female reproduction may be classified as alterations in female sexual maturation, effects on fertility and menstrual cycle, alterations in levels of female sex hormones, and changes in morphology or histology of female reproductive organs as well as the placenta.

5.4.4.2 Effects on embryo/fetal morphology

The 1986 Lead AQCD summarized numerous reports that found associations between prenatal exposure to high doses of Pb and increased incidences of teratogenic effects (particularly tail stunting) in rodents (Ferm and Carpenter, 1967, Wide, 1985). More recent studies provide additional support for the teratogenic effects of lead in experimental animals (Dey et al., 2001; Flora and Tandon, 1987; Ronis et al., 1996). However, the few studies (including those described in the 1986 Lead AQCD and more recent reports) that have demonstrated teratogenic effects of Pb exposure are confounded by maternal toxicity.

Table 5-5. Selected Studies Showing the Effects of Lead on Mammalian Embryogenesis and Development

Citation	Species/ Strain	Dose/Route/Form/Duration/Group size	Endpoint/Magnitude of Effect/p-value	Blood Lead Concentration (PbB)
Cory-Slechta et al. (2004)	Rat/Long-Evans	Lead acetate in drinking water (150 ppm); 2 months before breeding until the end of lactation; 14 rats no maternal stress with Pb exposure, 15 rats no maternal stress with Pb exposure, 18 rats maternal stress without Pb exposure, 23 rats maternal stress and Pb exposure	Pb alone (in male) ($p < 0.05$) and Pb plus stress (in females) ($p < 0.05$) permanently elevated corticosterene levels in offspring	PbB 30–40 µg/dL
Dearth et al. (2002)	Rat/Fisher 344	12 mg/mL Pb acetate gavage during gestation and lactation exposure 4 groups: control group, gestation and lactation exposure, gestation only exposure, lactation only exposure 10–32 litters per group (NOS)	Delayed onset of puberty ($p < 0.05$); suppressed serum levels of IGF_1, LH, and E_2 ($p < 0.001$); Pb altered translation and/or secretion of IGF_1 ($p < 0.001$).	Maternal PbB ~40 µg/dL Pups PbB as follows: Gest+lact ~38 µg/dL PND 10 Gest+lact ~15 µg/dL PND 21 Gest+lact ~3 µg/dL PND 30 Gest ~14 µg/dL PND 10 Gest ~3 µg/dL PND 21 Gest ~1 µg/dL PND 30 Lact ~28 µg/dL PND 10 Lact ~15 µg/dL PND 21 Lact ~3 µg/dL PND 30
Flora and Tandon (1987)	Rat/Albino (NOS)	Lead nitrate dissolved in water 2–20 mg/kg-d i.v. on day 9, 10, 11 of gestation; 6 rats in each group (0, 5, 10 , 20, 40 mg/kg lead)	Dose-dependant increase in external malformations at all doses ($p < 0.001$), particularly tail defects; dose-dependant decrease in number of live births at 20 and 400 mg/kg ($p < 0.001$); dose-dependent increase in number of resorptions per dam at ≤ 10 mg/kg ($p < 0.01$).	PbB 4.13 ± 0.61 µg/dL 0 mg/kg PbB 10.21 ± 0.61 µg/dL 5 mg/kg PbB 13.13 ± 0.27 µg/dL 10 mg/kg PbB 29.41 ± 0.41 µg/dL 20 mg/kg PbB 45.03 ± 0.31 µg/dL 40 mg/kg
Fox et al. (1991a)	Rat/Long-Evans hooded	Lactation exposure via dams exposed to 0.02 or 0.2% Pb in drinking water from PND 1 through weaning (PND 21); 8 female pups per litter (number of litter unspecified) control pups, 8 pups for litter (number of litter unspecified) low-level exposure pups, 8 pups per litter (number of litter unspecified) moderate level exposure pups	Long-term, dose-dependent decreases retinal Na/K ATPase activity in the female offspring (only female pups were used) (−11%; −26%) ($p < 0.05$).	PbB 18.8 µg/dL (0.02%) or 59.4 µg/dL (0.2%) at weaning

Table 5-5 (cont'd). Selected Studies Showing the Effects of Lead on Mammalian Embryogenesis and Development

Citation	Species/ Strain	Dose/Route/Form/Duration/Group Size	Endpoint/Magnitude of Effect/p-value	Blood Lead Concentration (PbB)
Fox et al. (1997)	Rat/Long-Evans hooded	0.02 or 0.2% Pb acetate in drinking water from PND 0–PND 21; 8 female pups per litter control pups; 8 pups per litter low-level exposure; 8 pups per litter moderate level exposure (number of litters per dose unspecified)	Developmental and adult Pb exposure for 6 weeks produced age and dose-dependent retinal degeneration such that rods and bipolar cells were selectively lost; at the ultrastructural level, all dying cells exhibit the classical morphological features of apoptotic cell death; decrease in the number of rods was correlated with the loss of rhodopsin content per eye confirming that rods were directly affected by Pb ($p < 0.05$); single-flash rod ERGs and cone ERGs obtained from lead-exposed rats demonstrated that there were age- and dose-dependent decreases in the rod a-wave and b-wave sensitivity and maximum amplitudes without any effect on cones; in adult rats exposed to Pb for three weeks, qualitatively similar ERG changes occurred in the absence of cell loss or decrease in rhodopsin content ($p < 0.05$); developmental and adult Pb exposure for three and six weeks produced age- and dose-dependent decreases in retinal cGMP phosphodiesterase (PDE) activity resulting in increased cGMP levels ($p < 0.05$); retinas of developing and adult rats exposed to Pb exhibit qualitatively similar rod mediated ERG alterations as well as rod and bipolar apoptotic cell death ($p < 0.05$). Similar biochemical mechanism such as the inhibition of rod and bipolar cell cGMP PDE, varying only in degree and duration, underlies both the lead-induced ERG rod-mediated deficits and the rod and bipolar apoptotic cell death ($p < 0.05$).	PbB weanlings 19 ± 3 (low exposure) or 59 ± 8 µg/dL (moderate exposure), adult 7 ± 2 µg/dL (at PND 90)
Iavicoli et al. (2003)	Mouse/Swiss	Lead acetate in food (0.02, 0.06, 0.11, 0.2, 2, 4, 20, 40 ppm); exposure began 1 day after mating until litter was 90 days old; one litter of mice exposed to each dietary concentration	Low-level Pb exposure (PbB 2–13 µg/dL) reduced red cell synthesis ($p < 0.05$); high-level exposure (PbB 0.6-2 µg/dL) enhanced red cell synthesis ($p < 0.05$).	PbB 0.6 to <2.0 µg/dL or >2.0–13 µg/dL

Table 5-5 (cont'd). Selected Studies Showing the Effects of Lead on Mammalian Embryogenesis and Development

Citation	Species/Strain	Dose/Route/Form/Duration/Group size	Endpoint/Magnitude of effect/p-value	Blood Lead Concentration (PbB)
Lögdberg et al. (1987)	Monkey/Squirrel	Lead acetate (5–20 mg/kg daily to maintain PbB) maternal dosing from 5–8.5 weeks pregnant to PND1 20 control; 11 lead-exposed monkeys	Increase in pre- and perinatal mortality among squirrel monkeys receiving Pb acetate p.o. during the last two-thirds of pregnancy (45% vs. 7–8% among controls). Statistically significant reductions in mean birth weight ($p < 0.05$) were observed in Pb exposed monkeys as compared to controls. Effects occurred without clinical manifestation of toxic effects in the mothers.	PbB 54 µg/dL (39–82 µg/dL)
Ronis et al. (1996)	Rat/Sprague-Dawley	0.6% Pb acetate in drinking water for various durations: PND 24–74 (pubertal exposure); PND 60–74 (post pubertal exposure); 11 males and females in pubertal exposure group (10 each in control pubertal group) 6 males and females post-pubertal exposure and control groups	Reduction in serum testosterone levels in male, not female; in female suppression of circulating E2 ($p < 0.05$) and LH ($p < 0.05$); reduction in male secondary sex organ weight ($p < 0.0005$); delayed vaginal opening and disrupted diestrous in females ($p < 0.005$); increased incidence of stillbirth (2% control vs. 19% Pb) ($p < 0.005$).	*In utero* PbB 250–300 µg/dL pre-pubertal PbB 30–60 µg/dL post pubertal PbB 30–60 µg/dL PbBs in the dams and offspring in this experiment were >200 µg/dL
Ronis et al. (1998a)	Rat/Sprague-Dawley	0.6% Pb acetate in drinking water *ad libitum* for various durations; GD 5 to PND 1; GD 5 to weaning; PND 1 to weaning; 3 control litters, 2 gestation exposure litters, 2 lactation exposure litters, 2 gestation and lactation exposure litters, 2 postnatal litters, 2 chronic litters (4 male and 4 female pups per litter)	Dose-dependent delay in sexual maturation (delayed vaginal opening) ($p < 0.0002$) following prenatal Pb exposure that continued until adulthood (85 days old); reduced birth weight ($p < 0.05$), more pronounced among male pups.	Group / Pup PbB Naïve / ~6 µg/dL Control / <2 µg/dL Gest / ~10 µg/dL Lac / ~3 µg/dL Gest+Lac / ~13 µg/dL Postnatal / ~260 µg/dL Chronic / ~287 µg/dL

Table 5-5 (cont'd). Selected Studies Showing the Effects of Lead on Mammalian Embryogenesis and Development

Citation	Species/ Strain	Dose/Route/Form/Duration/Group Size	Endpoint/Magnitude of Effect/p-value	Blood Lead Concentration (PbB)
Ronis et al. (1998b)	Rat/Sprague-Dawley	Lead acetate in drinking water (0.05% to 0.45% w/v); dams exposed until weaning; exposure of pups which continued until PND 21, 35, 55, or 85 5 control litters (0%), 10 low-dose litters (0.05%), 8 mid-dose litters (0.15%), 9 high-dose litters (0.45%) (4 male and 4 female pups per litter)	Prenatal Pb exposure that continues until adulthood (85 days old) delays sexual maturation in female pups in a dose-related manner ($p < 0.05$); birth weight reduced ($p < 0.05$), more pronounced among male pups; decreased growth rates ($p < 0.05$) in both sexes accompanied by decrease in plasma concentrations of IGF_1 through puberty ($p < 0.05$) and a significant increase in pituitary growth hormone during puberty ($p < 0.05$).	PbBs in the pups between the ages of 21 and 85 days were >100 µg/dL and reached up to 388 µg/dL
Ronis et al. (1998c)	Rat/Sprague-Dawley	Lead acetate 0.05, 0.15, or 0.45% in drinking water beginning GD 5 continuing until PND 21, 35, 55, or 85; 5 control litters (0%), 10 low-dose litters (0.05%), 8 mid-dose litters (0.15%), 9 high-dose litters (0.45%) (4 male and 4 female pups per litter)	Dose-responsive decrease in birth weight ($p < 0.05$), and crown-to-rump length ($p < 0.05$); dose-responsive delay in sexual maturity in male ($p < 0.05$) and female ($p < 0.05$); neonatal decrease in sex steroids ($p < 0.05$); pubertal decrease in testosterone (male) ($p < 0.05$), and E_2 (female) ($p < 0.05$); decrease estrous cyclicity at high dose ($p < 0.05$).	Dams: 0, 48, 88, or 181 µg/dL Pups PND 1: <1, ~40, ~70, or >120 µg/dL Pups PND 21: <1, >50, >160, or ~237 µg/dL Pups PND 35: <1, ~22, >70, or >278 µg/dL Pups PND 55: <1, >68, >137, or ~380 µg/dL Pups PND 85: <1, >43, >122, or >214 µg/dL
Ronis et al. (2001)	Rat/Sprague-Dawley	Lead acetate in drinking water to 825 or 2475 ppm *ad libitum* from GD 4 to GD 55 postpartum; 1 male and female pup/litter (5 litters per group) control group, 1 male and female pup/litter (5 litters per group) 825 ppm Pb acetate group, 1 male and female pup/litter (5 litters per group) 2475 ppm Pb acetate group	Dose-dependent decrease of the load of failure in male ($p < 0.05$); no difference in plasma levels of vitamin D metabolites; reduced somatic growth ($p < 0.05$), longitudinal bone growth ($p < 0.05$, and bone strength during the pubertal period ($p < 0.05$); sex steroid replacement did not restore skeletal parameters in Pb exposed rats; L-Dopa increased plasma IGF_1 concentrations, rates of bone growth, and bone strength measures in controls while having no effect in Pb exposed groups; DO gap x-ray density and proximal new endostreal bone formation were decreased in the distration gaps of the lead-treated animals ($p < 0.01$); distraction initiated at 0.2 mm 30 to 60 days of age.	PbB at 825 ppm was 67–192 µg/dL PbB at 2475 ppm was 120–388 µg/dL

Table 5-5 (cont'd). Selected Studies Showing the Effects of Lead on Mammalian Embryogenesis and Development

Citation	Species/ Strain	Dose/Route/Form/Duration/Group Size	Endpoint/Magnitude of Effect/p-value	Blood Lead Concentration (PbB)
	Rat/Sprague-Dawley	Lead in drinking water at 34 ppm from weaning of mothers through gestation and weaning of offspring until birth; 6 pups control group, 6 pups experimental group	Reduced body weight ($p = 0.04$); parotid function was decreased by nearly 30% ($p = 0.30$); higher mean caries scores than the control pups ($p = 0.005$); pre- and perinatal Pb exposure had significantly increased susceptibility to dental caries ($p = 0.015$).	PbB 48 ± 13 µg/dL

GMP, cyclic guanosine--3',5'-monophosphate; DO, distraction osteogenesis; E_2, estradiol; ERG, electroretinographic; GD, gestational day; IGF_1, insulin-like growth factor 1; LH, luteinizing hormone; NOS, not otherwise specified; PbB, blood Pb concentration; PDE, phosphodiesterase; PND, post-natal day

5.4.5 Effects on Growth and Endocrine Regulation of Growth

Studies conducted in rodents provide convincing evidence for an association between gestational Pb exposure and reduced birth weight and postnatal growth at doses that produce no clinical toxicity in the mothers (Dearth et al., 2002; Hamilton et al., 1994; Lögdberg et al., 1987; Piasek and Kostial, 1991; Pinon-Lataillade et al., 1995; Ronis et al., 1998a,b,c; Singh et al., 1993b; Watson et al., 1997). In squirrel monkeys, Lögdberg et al. (1987) reported a statistically significant reduction in mean birth weight following oral exposure to Pb acetate during the latter trimesters of pregnancy (mean maternal blood Pb 54 µg/dL [39 to 82 µg/dL]). Additional details concerning Lögdberg et al. (1987) are provided in Table AX5-4.3.

In addition, the literature provides convincing support for Pb-induced impairment of postnatal growth. Although some early studies (Minnema and Hammond, 1994; Hammond et al., 1993, 1990) ascribed the reduction in postnatal growth to reduced food consumption (suggesting an effect of Pb on the satiety endpoint), more recent studies report impaired growth unrelated to changes in food consumption. Ronis et al. (1996, 1998a,b,c) reported Pb-induced reductions in birth weight and postnatal growth that occurred in the absence of a significant alteration in food consumption. Han et al. (2000) found a reduction in the birth length of pups (pup blood Pb ~16 µg/dL on PND 1) whose mothers had been exposed to Pb up to 1 month before mating (maternal blood Pb on GD 9, 16, and 21 <40 µg/dL). Berry et al. (2002) reported depressed growth in rats exposed to lead for six weeks beginning at weaning, even though food consumption was higher in the lead-exposed rats.

Ronis et al. (2001) showed that in rats, pre- and postnatal (through PND 55) exposure to Pb reduced somatic longitudinal bone growth and bone strength during the pubertal period (blood Pb >67 µg/dL). These effects could not be reversed by stimulation of the growth hormone axis by supplemental sex hormone. These results suggest that Pb exposure may impair growth through a mechanism that involves a suppressed pituitary response to hypothalamic stimulation. The mechanism may be related to a reduction in plasma concentrations of IGF_1 following Pb exposure (Dearth et al., 2002; Ronis et al., 1998b). Dearth et al. (2002) exposed F344 rats to Pb by gavage beginning 30 days before mating and continuing until weaning of the pups at 21 days of age. By PND 30, all three groups had blood Pb ≤3 µg/dL and all lead-exposed groups exhibited decreased serum levels of IGF_1, LH, and E_2. Since liver IGF_1 mRNA was not affected, it appeared that Pb altered the translation and/or secretion of IGF_1, which in

turn decreased LH-releasing hormone at the hypothalamic level. Additional details concerning Dearth et al. (2002) are provided in Table AX5-4.3. An effect on IGF_1 also been demonstrated by Ronis et al. (1998b).

5.4.6 Effects on Other Endocrine Systems during Development

Recent experimental animal studies provide evidence for an interaction between Pb exposure and stress hormones, including glucocorticoids and catecholamines (Cory-Slechta et al., 2004; Yu et al., 1996; Vyskočil et al., 1991; Saxena et al., 1990). Lead has been reported to increase stress hormone levels (Vyskočil et al., 1991).

Cory-Slechta et al. (2004) reported a persistent effect of maternal Pb exposure (blood Pb 30-40 µg/dL) on corticosteroid levels in adult offspring. Both male and female offspring born to dams exposed to lead exhibited elevated corticosteroid levels as adults. In female offspring, the Pb effect was potentiated when maternal Pb exposure occurred in combination with environmental stress (administered as restraint). These data suggest that brief exposures to Pb during development may result in persistent changes in the hypothalamic-pituitary-adrenal axis (e.g., fetal glucocorticoid programming). Additional details concerning Cory-Slechta et al. (2004) are provided in Table AX5-4.3.

The interplay between Pb and stress hormones is consistent with the findings of Yu et al. (1996) wherein neonatal exposure to Pb (blood Pb 70 µg/dL) decreased cold-water swimming endurance (a standard test for stress endurance). The enhancement of Pb-induced toxicity by stress was also reported by Saxena et al. (1990) in adult male rats. Saxena et al. (1990) reported enhanced testicular injury when rats were exposed to immobilization stress in combination with Pb exposure (blood Pb >200 µg/dL).

5.4.7 Effects on Other Organ Systems during Development

5.4.7.1 Developmental Effects on Blood and Liver

Recent data provide evidence for Pb-induced alterations in developing hematopoietic and hepatic systems. However, the data concerning Pb exposure effects on the developing hematopoietic system are limited. The 1986 Lead AQCD proposed that alterations in blood ALAD activity and erythrocyte protoporphyrin were possible biomarkers for subtle, prenatal effects of Pb on heme synthesis (Hayashi 1983a,b; Jacquet et al., 1977; Prigge and Greve, 1977;

Hubermont et al., 1976). A more recent study (Iavicoli et al., 2003) of Pb effects on RBC production, Hb concentration, and Hct was not able to clearly establish a dose-response relationship for these endpoints. Although limited by small group size (one litter per dose), dietary exposure during conception, lactation, and through weaning to 90 days of age increased red blood cell synthesis, blood hemoglobin concentration, and hematocrit in offspring that had blood Pb levels in the range of 0.6 to 2 μg/dL; and decreased red cell blood cell synthesis, blood hemoglobin concentration, and hematocrit in offspring in that had blood Pb concentrations in the range of 2 to 13 μg/dL. More data are needed to clarify the effect of low-dose Pb exposure on blood endpoints.

Two rodent studies provide limited suggestive evidence that Pb exposure during development produces changes in hepatic enzymes and other biomarkers of hepatic function. Pillai and Gupta (2005) reported that long-term exposure of rats (pre-mating, gestation, and lactation) to Pb acetate (subcutaneous injections of 0.05 mg/kg-day; blood Pb not reported) resulted in reduced activities of maternal hepatic steroid (E_2) metabolizing enzymes (17-β-hydroxy steroid oxidoreductase and UDP glucuronyl transferase) and decreased hepatic CYP450 content. Corpas et al. (2002a) reported that exposure to Pb in drinking water exposure during gestation and lactation (pup blood Pb ~22 μg/dL at PND 12 and PND 21) resulted in alterations in the hepatic systems of neonates (PND 12) and pups (PND 21). The effects manifested as alterations in several biochemical indicators of hepatic toxicity: reductions in Hb, iron, alkaline and acid phosphatase levels, and hepatic glycogen, and elevated blood glucose. These data suggest that Pb may alter hepatic function during development; however, more data are needed to determine whether these effects are persistent.

5.4.7.2 Developmental Effects on Skin

Recent data provide limited evidence of altered soft tissue development resulting from Pb exposure. The literature includes one report of Pb-induced abnormalities in skin development. Dey et al. (2001) reported that the pups of mice exposed orally to Pb citrate (5 μg/kg-day) throughout gestation exhibited a variety of skin anomalies, including perforations, cell deformity, and disordered collagen bundles. Blood Pb levels of mothers and pups were not reported. Although detailed biochemical studies are required to elucidate the mechanism for structural

abnormalities, it appears that covalent binding of Pb ions to the sulfate group of glycosaminoglycans may be involved.

5.4.7.3 Developmental Effects on the Retina

Several studies have found that Pb exposure during early postnatal development impairs retinal development in female Long-Evans (LE) hooded rats (Fox et al., 1997, 1991a,b; Fox and Rubenstein, 1989; Fox and Chu, 1988). Of these, two studies are particularly important. Fox et al. (1991a) demonstrated that lactational exposure of LE hooded rats (blood Pb 18.8 or 59.4 μg/dL) resulted in long-term, dose-dependent decreases retinal Na/K ATPase activity in the female offspring (only female pups were used). Fox et al. (1997) subsequently demonstrated that lactational exposure to female LE hooded rats (blood Pb 19 ± 3 or 59 ± 8 μg/dL) or drinking water exposure to adult females (blood Pb 56 ± 9 μg/dL) resulted in differential age- and dose-dependent alterations in retinal structure and function following low (blood Pb <20 μg/dL) and moderate (blood Pb <60 μg/dL) Pb exposures during lactation or long-term (~60 days) exposure during adulthood. The mode of action for the effects of Pb on retinal development may be related to impaired Na/K ATPase activity (Fox et al., 1991a). The observation of reduced enzyme activity in the retina, but not in the kidney, suggests specificity for the retinal alpha-3 isozyme of Na/K ATPase, rather than the renal alpha-1 isozyme of Na/K ATPase. The authors suggested that this specificity may play a role in the target organ-specific toxicity of Pb (Fox et al., 1991a).

5.4.8 Summary

The 1986 Lead AQCD presented unequivocal evidence (derived principally from studies of rodents) for effects of Pb on reproduction and development in laboratory animals. This included evidence for lethal effects in developing organisms exposed to Pb during gestation and in the neonatal period, as well as a variety of sublethal effects on reproduction and development. Sublethal effects included changes in levels or function of reproductive hormones, effects on maturation of reproductive systems, persistent toxic effects on the gonads (both male and female), and adverse effects on the conceptus. More subtle effects on hormone metabolism and reproductive cell structure of developing organisms were also documented.

- More recent studies support earlier conclusions, presented in the 1986 Lead AQCD, that Pb can produce temporary and persistent effects on male and female reproductive function and development and that Pb disrupts endocrine function at multiple points along the hypothalamic-pituitary-gonadal axis.

- Studies conducted in male experimental animals unequivocally demonstrate that Pb exposure during early development (blood Pb >30 μg/dL) can delay the onset of puberty and alter reproductive function later in life.

- Persistent effects of Pb exposure on the male reproductive system may derive from disruption in pulsatile release of sex hormones during early development (Ronis et al., 1998c).

- Experimental animal studies provide convincing evidence that Pb acts as an endocrine disruptor in males at various points along the hypothalamic-pituitary-gonadal axis. Although there is evidence for a common mode of action, consistent effects on circulating testosterone levels are not always observed in Pb-exposed animals. The inconsistency in the reports of circulating testosterone levels complicates the derivation of a dose-response relationship for this endpoint.

- More recent studies in animals provide additional support for testicular damage (i.e., ultrastructural changes in testes and cytotoxicity in Sertoli cells) following exposure to Pb and demonstrated ultrastructural changes in testes of monkeys at blood Pb levels of 35 to 40 μg/dL. Lead-induced oxygen free radical generation is the plausible mechanism of testicular injury in primates and rodents.

- Recent studies of various mammalian species provide convincing support for Pb-induced endocrine-mediated alterations of the female reproductive system. The nonhuman primate studies provide dose-response information concerning the effects of Pb on female sex hormones and menstrual cycle.

- Exposures of monkeys to Pb resulting in chronic blood Pb levels <20 μg/dL produce few effects on circulating hormone levels and do not alter the menstrual cycle. Higher exposures of monkeys to Pb (blood Pb >40 μg/dL) alter circulating hormone levels and the menstrual cycle, with more marked changes in these endpoints occurring at higher blood Pb levels.

- Several modes of action for Pb-induced alterations in female reproduction have been proposed, including changes in hormone synthesis or metabolism and changes in hormone receptor levels. In addition, Pb may alter sex hormone release and imprinting during early development.

- More recent studies have confirmed that Pb exposure disturbs female fertility; however, Pb exposure does not generally produce total sterility.

- Studies in nonhuman primates and rodents have also demonstrated reductions in litter size, implantation dysfunction, and decreased postnatal survival following Pb exposure of gravid female experimental animals (blood Pb >30 μg/dL).

- Lead-induced changes in morphology or histology in female sex organs and placenta may explain reduced fertility and impaired female reproductive success.

- Exposure to Pb in early pregnancy also produces structural changes in the epithelium of the uterus of mice. These changes in uterine tissue may impair successful implantation of the blastocysts.

- Histological and morphological effects on the uterus and placenta may explain the reduced birth weight that has been associated with prenatal Pb exposure (possibly due to placental insufficiency).

- Pre- and postnatal exposure to Pb has been demonstrated to result in fetal mortality and produce a variety of sublethal effects in the offspring. Many of these Pb-induced sublethal developmental effects occur at maternal blood Pb levels that do not result in clinical (overt) toxicity in the mothers. The few studies that have reported teratogenic effects resulting from Pb exposure are confounded by maternal toxicity.

- Studies conducted in rodents and primates provide convincing evidence for an association between Pb exposure and reduced birth weight and postnatal growth at doses that produce no clinical toxicity in the mothers (maternal blood Pb >40 µg/dL).

- Recent experimental animal studies provide evidence for an interaction between Pb exposure during development (blood Pb 30 to 40 µg/dL) and stress hormones, including both glucocorticoids and catecholamines.

- Lead exposure during early postnatal development (blood Pb ~20 µg/dL) impairs retinal development in female Long-Evans hooded rats.

- In addition, recent studies provide limited evidence for Pb-induced alterations in developing skin, and hematopoietic and hepatic systems.

5.5 CARDIOVASCULAR EFFECTS OF LEAD

5.5.1 Introduction

Numerous large and small epidemiological studies have attempted to examine the link between Pb exposure and development of hypertension (HTN) in the general population and occupationally exposed individuals. In addition, a number of studies have reported on other Pb-associated cardiovascular effects in Pb-exposed humans (U.S. Environmental Protection Agency, 1990). While several studies have demonstrated a positive correlation between blood pressure and blood Pb concentration, others have failed to show such association when controlling for confounding factors such as tobacco smoking, exercise, body weight, alcohol

consumption, and socioeconomic status. Thus, the studies that have employed blood Pb level as an index of exposure have shown a relatively weak association with blood pressure. In contrast, the majority of the more recent studies employing bone Pb level have found a strong association between long-term Pb exposure and arterial pressure (Chapter 6). Since the residence time of Pb in the blood is relatively short but very long in the bone, the latter observations have provided rather compelling evidence for a positive relationship between Pb exposure and a subsequent rise in arterial pressure. This section reviews the published studies pertaining to the cardiovascular effects of Pb exposure in experimental animals, isolated vascular tissues, and cultured vascular cells.

5.5.2 Lead Exposure and Arterial Pressure in Experimental Animals

Numerous studies have shown that exposure to low levels of Pb for extended periods results in a delayed onset of arterial HTN that persists long after the cessation of Pb exposure in genetically normal animals (see Tables AX5-5.1 to AX5-5.5). In addition, Pb exposure during gestation has been reported to significantly increase arterial pressure in the third trimester of pregnancy in SD rats given a low calcium diet (Bogden et al., 1995). Taken together, these observations provide irrefutable evidence that extended exposure to low levels of Pb can result in the subsequent onset of HTN in experimental animals.

Many studies have been conducted to explore the mechanisms by which chronic Pb exposure may cause HTN. Most of these studies have examined various blood-pressure regulatory and vasoactive systems in animal models of Pb-induced HTN. In addition, several studies have investigated the direct effect of Pb on vascular tone or the ability of Pb to modify the response to vasoconstrictor/vasodilator agents in isolated vascular tissues. Finally, a number of studies have explored the effect of Pb on cultured endothelial and vascular smooth muscle cells. An overview of the findings of these studies is provided below.

5.5.2.1 Effect of Lead on Production of Reactive Oxygen Species and Nitric Oxide Metabolism

Reactive oxygen species (ROS), such as superoxide (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) are normally produced in the course of metabolism and are safely contained by the natural antioxidant defense system. Excess production and/or diminished

containment of ROS can lead to oxidative stress in which uncontained ROS can attack and denature functional/structural molecules and, thereby, promote tissue damage, cytotoxicity, and dysfunction. In fact, oxidative stress has been implicated in the pathogenesis of HTN, atherosclerosis, neurodegenerative disorders, aging, and neoplasm among other afflictions. During the past decade, several studies have demonstrated that Pb exposure causes oxidative stress, particularly in the kidney and cardiovascular tissues, as well as in cultured endothelial and vascular smooth muscle cells (VSMC). The in vivo studies have further shown that Pb-induced oxidative stress is, at least in part, responsible for the associated HTN in experimental animals. Relevant published studies pertaining to this issue are summarized below and listed in Annex Table AX5-5.1.

Khalil-Manesh et al. (1994) were among the first to suggest that oxidative stress may be involved in the pathogenesis of Pb-induced HTN. This assumption was based on the observation that chelation therapy with dimethyl succinic acid (DMSA) rapidly ameliorated HTN and raised plasma cGMP level in rats with Pb-induced HTN. They further demonstrated that DMSA possesses strong antioxidant properties in vitro. Accordingly, they theorized (a) that Pb exposure may increase the generation of ROS, which, in turn, elevate arterial pressure by reacting with and inactivating endothelium-derived-relaxing factor (EDRF), and (b) that by scavenging ROS, DMSA rapidly lowers blood pressure prior to significantly affecting body Pb burden.

In a subsequent study, Gonick et al. (1997) showed a marked increase in renal tissue content of lipid peroxidation product malondialdehyde (MDA) coupled with significant upregulations of endothelial (eNOS) and inducible (iNOS) nitric oxide synthases. Thus, the study provided evidence for the occurrence of oxidative stress and compensatory upregulation of NOS isotypes in the kidney of animals with Pb-induced HTN.

In another study, Ding et al. (1998) showed that infusion of NOS substrate, L-Arginine, lowers blood pressure to a much greater extent in rats with Pb-induced HTN than that seen in either control animals or DMSA-treated Pb-exposed animals. The data, therefore, provided indirect evidence for the role of depressed NO availability in the pathogenesis of Pb-induced HTN. The study further suggested that oxidative stress may be responsible for diminished NO availability in this model. It should be noted that administrating cell-impermeable native SOD did not lead to a further reduction of blood pressure beyond that seen with L-Arginine alone. As with the previous study (Khalil-Manesh 1994), oral DMSA therapy for 2 weeks significantly

lowered blood pressure in the Pb-exposed animals. This was accompanied by a significant reduction of blood Pb concentration. In an attempt to explore whether the observed amelioration of Pb-induced HTN was due to the reduction of Pb burden or alleviation of oxidative stress by DMSA, Vaziri et al. (1997) carried out a study in which rats with Pb-induced HTN were treated with a lazaroid compound, a potent, non-chelating antioxidant. The study revealed marked elevation of blood pressure and oxidative stress (increased lipid peroxidation) and reduced NO availability (depressed urinary NO_2 + NO_3 excretion) in the untreated rats with Pb-induced HTN. Antioxidant therapy with the lazaroid compound resulted in a significant alleviation of oxidative stress, improved NO availability, and a marked attenuation of HTN without affecting blood Pb concentration. Thus, the latter study provided convincing evidence for the role of oxidative stress as a major mediator of Pb-induced HTN. The study further demonstrated that Pb-induced HTN is associated with diminished NO availability and that the latter was mediated by oxidative stress. The reduction in NO availability observed in rats with Pb-induced HTN (Pb acetate, 100 ppm in drinking water for 12 weeks) was recently confirmed by Dursun et al. (2005) in rats treated with daily IP injection of Pb acetate (8 mg/Kg) for 2 weeks. The authors showed that the increase in arterial pressure was accompanied by a significant reduction of urinary NO_2 + NO_3 excretion and a significant fall in renal blood flow (indicating increased renal vascular resistance), mimicking the effect of the NOS inhibitor LNAME.

To further explore the cause for the observed reduction of NO availability, Vaziri et al. (1999a) subsequently studied the expression of eNOS and iNOS in the kidney and cardiovascular tissues of rats with Pb-induced HTN. The study showed that the reduction in NO availability is paradoxically associated with a significant upregulation of NOS isotypes. Moreover, in vitro incubation experiments revealed no significant change in NOS activity in the presence of lead. Interestingly, antioxidant therapy with pharmacological doses of vitamin E and ascorbic acid reversed the upregulation of NOS isotypes and paradoxically raised NO availability in the subgroup of rats with Pb-induced HTN (Vaziri et al., 1999a). These observations were subsequently confirmed by Vaziri and Ding (2001) who showed marked reduction of NO availability despite significant upregulations of eNOS, nNOS, and iNOS in the aorta, heart, kidney, and brain of rats with Pb-induced HTN and their normalization with the administration of superoxide-scavenger tempol (15 mg/Kg IP/day) for 2 weeks. It is noteworthy that tempol administration had no effect on the measured parameters in the control animals. Taken together,

these observations indicated that ROS-mediated NO inactivation and, hence, depressed NO availability, results in a compensatory upregulation of NOS isotypes in animals with Pb-induced HTN. This phenomenon is consistent with other studies from this group, which have demonstrated the presence of a negative-feedback regulation of eNOS by NO (Vaziri and Wang, 1999; Vaziri et al., 2005).

The occurrence of compensatory upregulation of NOS by oxidative stress in Pb-exposed intact animals described above was subsequently replicated by Vaziri and Ding (2001) in cultured human endothelial cells incubated in media containing different concentrations of Pb acetate (versus control media containing sodium acetate). Once again, co-incubation with tempol prevented this phenomenon. This study confirmed the ability of Pb to affect endothelium independently of its effects on humoral or hemodynamic factors, which are operative in vivo. Taken together, these observations suggest that Pb-induced reduction of biologically active NO is not due to the reduction of NO-production capacity. Instead, it is linked to oxidative stress. In an attempt to explore this supposition, in a separate study, Vaziri et al. (1999b), tested the hypothesis that avid inactivation and sequestration of NO by ROS may be, in part, responsible for the reduction of NO availability in animals with Pb-induced HTN. To this end, they tested for the presence of immunodetectable nitrotyrosine in kidney, brain, and cardiovascular tissues harvested from untreated and antioxidant-treated (vitamin E + vitamin C) rats with Pb-induced HTN and normal control rats. Nitrotyrosine was used as a marker of NO oxidation by ROS ($NO + O_2 \rightarrow ONOO^-$, $ONOO^-$ + tyrosine → nitrotyrosine). The study showed an overabundance of nitrotyrosine in all plasma and tested tissues in the untreated rats with Pb-induced HTN. Antioxidant therapy reduced nitrotyrosine abundance, attenuated HTN, and simultaneously raised NO availability in the subgroup of rats with Pb-induced HTN but had no effect on the normal control group. These observations provided compelling evidence that Pb-induced HTN causes oxidative stress, which, in turn, promotes functional NO deficiency via ROS-mediated NO inactivation. The latter, in turn, participates in the development and maintenance of HTN and cardiovascular abnormalities. In addition, the formation of the highly cytotoxic reactive nitrogen species, peroxynitrite ($ONOO^-$), from the NO-ROS interaction and the associated nitrosative stress could potentially contribute to the long-term cardiovascular, renal, and neurological consequences of Pb exposure.

In subsequent studies, Vaziri et al. (2003) explored the expression of NAD(P)H oxidase (which is a well-recognized source of ROS in, not only, the immune cells but also in renal, cardiovascular, and neuronal tissues) in animals with Pb-induced HTN. In addition, expression of the main antioxidant enzymes, namely Mn and CuZn-superoxide dismutases (SOD), catalase and glutathione peroxidase were investigated. The study revealed significant upregulation the $gp91^{phox}$ subunit of NAD(P)H oxidase in the brain as well as a trend for higher levels in the renal cortex and left ventricle of rats with Pb-induced HTN. This was accompanied by a significant compensatory upregulation of CuZn SOD in the kidney and brain, and of Mn SOD in the heart, of rats with Pb-induced HTN. In contrast, despite the presence of oxidative stress, catalase and glutathione peroxidase activity levels were unchanged. In a more recent study, Farmand et al. (2005) showed a significant increase in CuZn SOD activity with no change in either catalase or glutathione peroxidase activity in the aorta of rats with Pb-induced HTN compared with control animals. Since the latter enzymes are responsible for the reduction of H_2O_2 and lipoperoxides, the lack of an appropriate rise in their tissue levels may contribute to the severity of oxidative stress in Pb-exposed animals.

The contribution of oxidative stress in the pathogenesis of HTN in this model was confirmed by experiments that demonstrated normalization of arterial pressure with the infusion of superoxide-scavenger, tempol, in rats with Pb-induced HTN (but no change was observed in the blood pressure in the control rats) (Vaziri et al., 2003). As noted above, the relative reduction of tissue catalase and glutathione peroxidase, which are responsible for the reduction of H_2O_2 to water and molecular oxygen $(2H_2O_2 \xrightarrow[\text{GPX}]{\text{CAT}} 2H_2O + O_2)$, can result in accumulation of H_2O_2. H_2O_2 serves as a cellular growth signal, as well as a substrate for hydroxyl radical ($^{\cdot}OH$) generation. The former action can potentially contribute to cardiovascular remodeling, whereas the latter can promote oxidative injury. In a recent study, Ni et al. (2004) demonstrated a transient rise in O_2^- production followed by a sustained rise in H_2O_2 production by human coronary endothelial and vascular smooth muscle cells cultured in media containing Pb acetate versus the control media containing Na-acetate. This was accompanied by, and primarily due to, upregulation of NAD(P)H oxidase and SOD together with reduced or unchanged catalase and glutathione peroxidase levels. Accordingly, the results of this in vitro study confirmed the findings of the in vivo studies and validated the anticipated accumulation of H_2O_2.

As noted above, H_2O_2 is the substrate for the Fenton and Haber-Weiss reactions, which culminate in formation of the highly cytotoxic·OH ($H_2O_2 + e^- \rightarrow OH + OH^-$). Thus, the accumulation of H_2O_2 in animals with Pb-induced HTN can facilitate·OH production and, thereby, promote oxidative stress and tissue injury. This supposition was confirmed in a series of studies by Ding et al. (2001), who showed increased hydroxyl radical production in rats with Pb-induced HTN. Oxidative stress, HTN, and excess hydroxyl radical production were all reversed with IV infusion of the reputed hydroxyl radical scavenger, DMTU, in the Pb-exposed animals. Increased hydroxyl radical production observed in intact animals with Pb-induced HTN was confirmed in Pb-treated cultured endothelial cells (Ding et al., 2000). The role of oxidative stress in the pathogenesis of HTN and endothelial dysfunction (depressed NO availability) has been substantiated by a number of other investigators. For instance, Attri et al. (2003) showed that exposure to Pb for up to 3 months resulted in a significant rise in arterial pressure, which was substantially ameliorated by coadministration of the antioxidant vitamin ascorbic acid (20 mg/rat) in Wistar-Kyoto rats. The rise in arterial pressure in Pb-treated rats was accompanied by diminished NO availability (low plasma $NO_2 + NO_3$) and biochemical evidence of oxidative stress, i.e., elevations of plasma MDA, a DNA oxidation product (8-hydroxyguanosine), and diminished ferric-reducing antioxidant power, as well as electrophoretic evidence of DNA damage. Amelioration of HTN by antioxidant therapy was accompanied by improved NO availability (plasma $NO_2 + NO_3$), marked attenuation of oxidative stress, and partial reduction of DNA damage in this model. In another study, Malvezzi et al. (2001) showed partial amelioration of HTN in Pb-exposed rats with the administration of either DMSA or L-arginine and showed a much greater response with the combination thereof. These observations support the role of interaction of ROS and NO in the pathogenesis of Pb-induced HTN in the rat.

As cited above, Pb-induced HTN is associated with and is, at least in part, due to ROS-mediated inactivation and hence, reduced availability of biologically active NO. Many of the biological actions of NO are mediated by cGMP, which is produced from the substrate GTP by the cytosolic enzyme soluble guanylate cyclase (sGC). sGC is expressed in VSMC and several other cell types. The enzyme is activated by NO to produce cGMP, which, in turn, promotes vasorelaxation by lowering cytosolic Ca^{2+} concentrations. In an earlier study, Khalil-Manesh et al. (1993a) demonstrated a significant reduction of plasma and urinary cGMP in rats with Pb-induced HTN. These observations prompted a number of studies to

evaluate the effect of Pb on sGC expression and cGMP production in vascular tissues obtained from rats with Pb-induced HTN or in normal vascular tissues incubated in Pb-containing media. For instance, Marques et al. (2001) found significant reductions of acetylcholine- and Na-nitroprusside-induced vasorelaxation, despite upregulation of eNOS, in the aorta of rats with Pb-induced HTN. This was associated with marked downregulation of sGC abundance and diminished cGMP production in the aorta. In an attempt to explore the possible role of oxidative stress in Pb-induced downregulation of sGC, they included a group of rats that were co-treated with Pb and the antioxidant vitamin ascorbic acid. Antioxidant therapy ameliorated HTN, restored vasorelaxation response to acetylcholine and Na-nitroprusside, and normalized sGC expression and cGMP production. The authors, therefore, identified diminished sGC as another mechanism by which Pb exposure can promote endothelial dysfunction and HTN. They further showed that Pb-induced downregulation of sGC is mediated by oxidative stress, as evidenced by its prevention with antioxidant therapy. Downregulation of sGC protein abundance in the aorta of Wistar rats with Pb-induced HTN was recently confirmed by Farmand et al. (2005) in the Pb-exposed Sprague-Dawley rats. In another study, Courtois et al. (2003) showed that 24-h incubation of normal rat aorta in the Pb-containing media resulted in a concentration-dependent downregulation of sGC (beta subunit), with the maximum effect observed at 1 ppm concentration. This was associated with increased O_2^- production and upregulation of cyclooxygenase-2 (COX-2) expression. Co-incubation with ascorbic acid reduced COX-2 expression and O_2^- production and attenuated, but did not fully prevent, the Pb-induced downregulation of sGC. Similarly, addition of COX-2 inhibitor Rofecoxib or of protein kinase A inhibitor (H-89) partially mitigated the Pb-induced downregulation of sGC in vitro. However, the COX-2 inhibitor failed to reduce O_2^- production in Pb-exposed vascular tissues. Based on these observations, the authors concluded that Pb exposure downregulates vascular tissue sGC abundance via induction of oxidative stress and upregulation of COX-2.

Oxidative stress and altered NO metabolism can potentially trigger a cascade of events that work in concert to promote HTN and cardiovascular disease in Pb-exposed organisms. Some of these potential links are illustrated in Figure 5-8.

5.5.2.2 Protein Kinase C, Inflammation, NFκB Activation, and Apoptosis

Protein kinase C (PKC) isoforms belong to a family of serine-threonine kinases, which

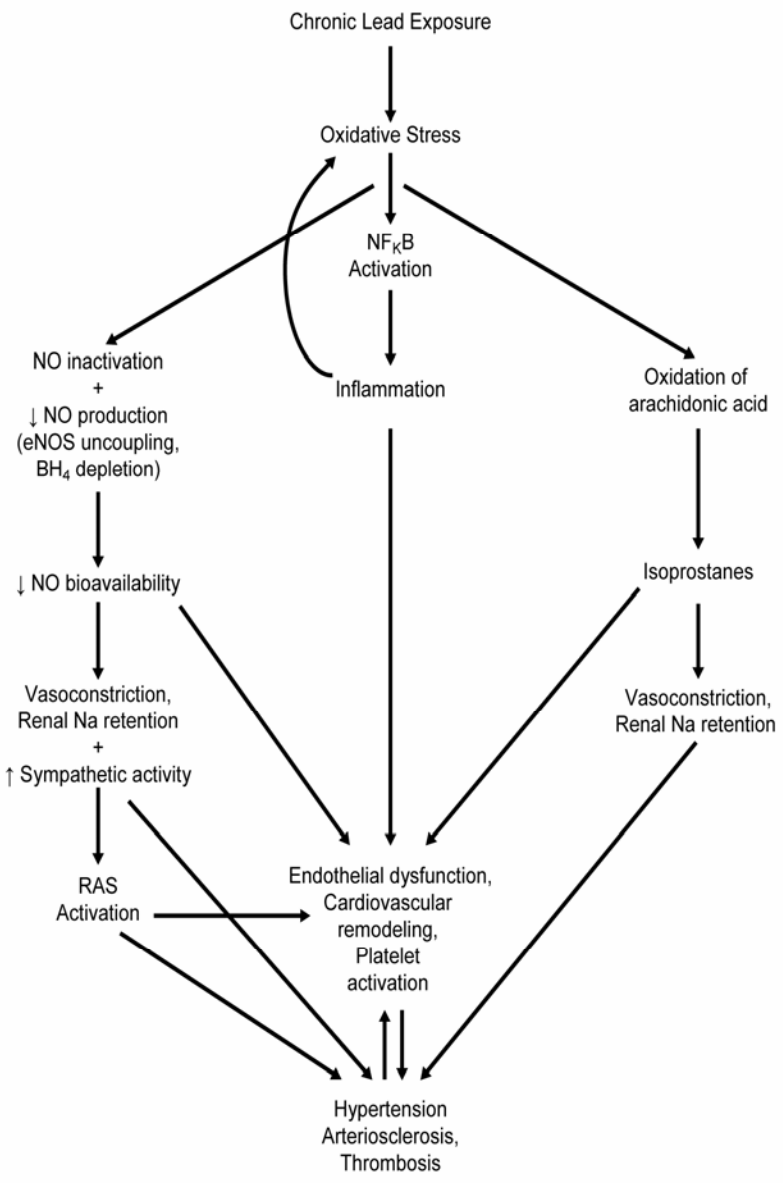

Figure 5-8. This illustration depicts some of the potential mechanisms by which oxidative stress may participate in the pathogenesis of lead-induced HTN and cardiovascular complications. In the presence of oxidative stress, uncontained reactive oxygen species (ROS) inactivate nitric oxide (NO), deplete NO synthase cofactor (tetrahydrobiopterin), uncouple eNOS, promote generation of isoprostanes by oxidizing arachidonic acid, and activate the redox-sensitive transcription factor NFκB. Together, these events can cause vasoconstriction, salt retention, sympathetic system activation, renin-angiotensin system stimulation, platelet adhesion, and, thereby, endothelial dysfunction, hypertension (HTN), inflammation, arteriosclerosis, and thrombosis.

serve numerous diverse cellular functions. For instance, PKC is involved in regulating vascular contractility, blood flow, permeability, and cell growth. In this regard, the activation of PKC has been shown to cause vascular contraction and Pb exposure has been found to raise PKC activity.

For example, Hwang et al. (2002) found increased PKC activity in the erythrocytes of a group of Pb-exposed Korean workers, and Markovac and Goldstein (1988b) showed a significant increase in PKC activity in rat brain micro vessels following exposure to micromolar Pb concentrations. Also, Watts et al. (1995) demonstrated that Pb acetate (10^{-10} to 10^{-3} M) caused contraction in an isolated rabbit mesenteric artery preparation. This Pb-induced vasoconstriction was unaffected by denudation of endothelium, while it was significantly potentiated by PKC agonists and attenuated by a PKC inhibitor. Calcium channel blockade with verapamil attenuated, but did not abolish, Pb-induced vasoconstriction. These findings were considered to indicate that activation of PKC is, in part, responsible for Pb-induced vasoconstriction, independently of endothelium or extracellular influx of calcium. Taken together, these observations suggest that the activation of PKC in the vascular smooth muscle cells may, in part, contribute to the pathogenesis of Pb-induced HTN by enhancing vascular contractility. It should be noted, however, that Pb-induced contraction has been shown to be unaffected by a PKC inhibitor in rat aorta rings (Valencia, 2001). Thus, the contribution of PKC activation to the Pb-induced alteration of vascular contractility appears to be both vessel- and species-specific. It is of note that at high concentrations, Pb can reduce PKC activity in certain cell types, including mouse macrophages and rat brain cortex (reviewed by Watts et al. [1995]).

As noted earlier, Pb exposure results in oxidative stress in cultured VSMC and endothelial cells, as well as in intact animals. Oxidative stress can promote the activation of the nuclear transcription factor kappa B (NFκB) and, thereby, trigger inflammation and apoptosis. In this context, Ramesh et al. (2001) showed that exposure to low Pb levels (50 ppm in drinking water) for 90 days activates NFκB and capsases in the rat brain. It is of note that several studies have revealed the presence of renal tubulointerstitial infiltration of activated T cells, macrophages, and angiotensin II (Ang-II) producing cells in various forms of genetic and acquired HTN in experimental animals. Moreover, the associated tubulointerstitial inflammation has been shown to contribute to the pathogenesis of HTN in these disorders (Rodríguez-Iturbe, 2004). These abnormalities are accompanied by activation of the redox-sensitive NFκB, which can account for the associated inflammation (reviewed by Rodríguez-Iturbe et al. [2004]). The NFκB activation,

the accompanying inflammation, and HTN are ameliorated by antioxidant therapy in these models, pointing to the role of oxidative stress in this process. In a recent study, Rodríguez-Iturbe, et al. (2005) observed marked activation of NFκB coupled with tubulointerstitial accumulation of activated T-cells, macrophages, and Ang-II-producing cells, as well as increased apoptotic cells in the kidneys of Pb-exposed rats (100 ppm Pb acetate in water for 3 months). This was associated with increased nitrotyrosine staining (a marker of NO/ROS interaction) in the kidney tissue. Since tubulointerstitial inflammation plays a crucial role in the pathogenesis of HTN in various other models of HTN, its presence in the Pb-exposed animals may contribute to the associated HTN. Inflammation in Pb-induced HTN is not limited to the kidney. In fact, lymphocyte infiltration is reported in the periaortic tissues in rats with Pb-induced HTN (Carmignani et al., 2000). The inflammatory response to Pb exposure in the renal and vascular tissues outlined above parallels observations reported for the immune system in Section 5.9 of this chapter.

5.5.2.3 Effect of Lead Exposure on the Adrenergic System

The adrenergic system plays an important role in regulating arterial pressure, renal and systemic hemodynamics, and cardiac function in health and disease. For this reason, a number of clinical and animal studies have focused on the sympathetic system as a possible mediator of Pb-induced HTN and cardiovascular abnormalities. For instance, in a study of a group of Pb-exposed workers, Chang et al. (1996) found elevated plasma norepinephrine (NE), but normal plasma dopamine and epinephrine, levels. The constellation of these biochemical abnormalities points to increased sympathetic nervous system activity in Pb-exposed humans. The impact of Pb exposure on the sympathetic nervous system activity has been substantiated in experimental animals. For example, Chang et al. (1997) showed that administration of Pb (Pb acetate 0.5% in drinking H_2O) for 2 months resulted in significant rises in arterial pressure and plasma NE (but not epinephrine) in Wistar rats. This was coupled with significant reductions of the aorta β adrenergic receptor density and isoproterenol (β agonist)-stimulated cAMP production. In a subsequent study, Tsao et al. (2000) reported a significant rise in plasma NE coupled with marked reductions of β receptor density as well as diminished basal and isoproterenol-stimulated cAMP productions in the aorta and heart of Wistar rats with Pb-induced

HTN. In contrast to the heart and aorta, β receptor density as well as basal and β agonist-stimulated cAMP production were increased in the kidneys of Pb-exposed animals.

In another study, Carmignani et al. (2000) found significant elevations of blood pressure, plasma catecholamines, and cardiac contractility (dP/dt), together with reduced carotid blood flow in rats with Pb-induced HTN. The effect of Pb on the sympathetic nervous system activity was examined by Lai et al. (2002) who tested the rapid response to intrathecal (IT) injection of $PbCl_2$ in vivo and its addition to the thoracic cord slices in vitro in the rats. They found significant rises in arterial pressure and heart rate with IT injection of Pb-chloride. These effects of Pb were abrogated by the administration of ganglionic blockade using hexomethonium. The in vitro studies revealed a significant rise in excitatory and significant fall in inhibitory post-synaptic potentials with the addition of Pb to the bathing medium and their reversal with saline washout.

In a recent study, Chang et al. (2005) showed a gradual decline in blood, kidney, heart, and aorta Pb contents toward the control values within 7 months following cessation of exposure in rats with Pb-induced HTN. This was coupled with a parallel declines in arterial pressure, plasma NE and renal tissue β receptor density as well as parallel rises in the aorta and heart β receptors densities during the 7-month period following cessation of Pb exposure. However, while HTN and β receptor abnormalities were significantly improved, they were not completely reversed. It should be noted that bone Pb contents were not measured in this study and were most likely elevated despite normalization of blood and soft tissue levels. These findings provided evidence for the stimulatory effect of Pb on the sympathetic nervous system and for its contribution to the cardiovascular effects of Pb exposure.

5.5.2.4 Effects of Lead on the Renin-Angiotensin-Aldosterone (RAAS) and Kininergic Systems

The available data on the effects of Pb exposure on the RAAS are contradictory. This appears to be primarily due to variability in the dosage and duration of Pb exposure, as well as the age at which exposure is initiated or the animals studied. In addition, when present, nephropathy can potentially affect the RAAS profile of Pb-exposed animals or humans. The majority of animal studies of the effects of Pb on RAAS were conducted and published in the late 1970s and 1980s. In a meta-analysis of the studies published in that period, Vander (1988)

found increased plasma renin activity and renal tissue renin content in young rats after several weeks of Pb exposure sufficient to achieve blood Pb levels in the range of 30 to 40 μg/dL. Similar results were found in rats exposed to Pb in utero and for 1 month after birth. In contrast, plasma renin activity and renal renin contents were generally unchanged or even reduced in older rats whose Pb exposure had commenced in utero.

In a more recent study, Carmignani et al. (1999) showed a significant increase in plasma angiotensin converting enzyme (ACE) activity in the rats exposed to Pb (60 ppm Pb acetate in water) for 10 months beginning at an early age (weaning). This was accompanied by a significant increase in plasma kininase II, kininase I, and kallikrein activities. In a subsequent study, Sharifi et al. (2004) examined plasma and tissue ACE activity in young adult rats (weighing 200 g) exposed to Pb (100 ppm Pb acetate) for 2 to 8 weeks. They found significant rises in plasma, aorta, heart, and kidney ACE activities, peaking at 2 to 4 weeks. This was followed by a decline in plasma and tissue ACE activity to subnormal values by 8 weeks, at which point arterial pressure was markedly elevated. The authors concluded that the elevated ACE activity is involved in the induction of HTN but may not be necessary for maintaining HTN in Pb-exposed animals. Finally, in a recent study, Rodríguez-Iturbe et al. (2005) demonstrated a marked increase in the number of Ang-II positive cells in the kidneys of rats treated with Pb acetate (100 ppm in water) for 3 months. This observation points to heightened intra-renal Ang-II generation in rats with Pb-induced HTN.

Taken together, the data point to activation of the RAAS at some point in the course of Pb-induced HTN. Further studies are needed to fully elucidate the effects of Pb exposure on various other RAAS components.

5.5.3 Effects of Lead Exposure on Vasomodulators

In a study of a group of Pb workers with elevated blood Pb concentration, Cardenas et al. (1993) found a significant increase in urinary excretion of the metabolite of vasoconstrictive prostaglandin, thromboxan (TXB_2), and significant reduction of the vasodilatory prostaglandin, 6-keto-PGF1, when compared with the control workers. Subsequently, Hotter et al. (1995) confirmed the elevation of urinary TXB_2 in another group of Pb-exposed workers. Based on these observations, the authors suggested that Pb can alter the balance between vasoconstrictive and vasodilatory prostaglandins in a way that may contribute to HTN and cardiovascular disease.

In an attempt to examine such possible effects of Pb exposure in experimental animals, Gonick et al. (1998) measured urinary excretion of the above metabolites in the rat model of Pb-induced HTN. The study showed no significant difference in urinary excretion of the given prostaglandin metabolites between the Pb-exposed and control rats. However, in a recent in vitro study, Dorman and Freeman (2002) demonstrated that Pb promotes the release of arachidonic acid by vascular smooth cells via activation of phospholipase A_2. They further showed that, at low concentrations, Pb augments Ang-II-induced VSMC proliferation, whereas at a high concentration it reduces viability and cell count in unstimulated cells and reduces DNA synthases in Ang-II and fetal calf serum (FCS)-stimulated VSMC. Thus, Pb can increase the release of arachidonic acid (the substrate for prostaglandins) via activation of phospholipase A_2.

Given the limited and contradictory nature of the published data, further in-depth studies are needed to clarify the effects of Pb on regulation of arachidonic acid metabolism and the synthesis of various classes of prostaglandins.

Endothelin

Endothelins (ET) represent a family of potent vasoconstrictive peptides that are produced by endothelium and a number of other cell types. Excess production or increased sensitivity to ET can raise arterial pressure. In an attempt to explore the possible contribution of ET to the pathogenesis of Pb-induced HTN, Khalil-Manesh et al. (1993a) studied the effects of exposure to low and high levels of Pb (100 ppm versus 5000 ppm) in the drinking water for 1 to 12 months in rats. Rats exposed to low (but not high) levels of Pb exhibited HTN and a significant increase in plasma ET-3 concentration. These findings were confirmed by these investigators in a subsequent study of rats with Pb-induced HTN (Khalil-Manesh et al., 1994). Similarly, Gonick et al. (1997) demonstrated a significant elevation of plasma concentration and urinary excretion of ET-3 in rats with Pb-induced HTN. Courtois et al. (2003) showed that incubation in the Pb-containing media resulted in the downregulation of soluble guanylate cyclase and cGMP production in the isolated artery segment of normal rats. They further found that co-incubation with an ET-A receptor antagonist can partially reverse this effect of Pb. These findings suggest that the adverse effect of Pb exposure on cGMP production in the vascular tissue is, in part, mediated by its ability to raise ET activity. It, thus, appears that exposure to low-levels of Pb can raise activity or production of ET, which can, in turn, play a part in the pathogenesis of Pb-

induced HTN in the rat. Further studies are required to carefully explore the effects of Pb on various components of the ET system.

Atrial Natriuretic Factor

Atrial natriuretic factor (ANF) is produced and secreted by cardiac myocytes. Plasma concentration of ANF rises with volume expansion and declines with volume contraction. ANF serves as a vasodilator and a natriuretic agent and, as such, plays a role in regulating blood volume, vascular resistance, and, hence, arterial pressure. Giridhar and Isom (1990) measured ANF in rats treated with IP injection of Pb acetate (0.0 to 1.0 mg/kg/twice weekly for 30 days). The Pb-exposed animals exhibited fluid retention, which was coupled with a paradoxical dose-dependent decline in plasma ANF concentration. Based on these findings, they suggested that Pb may interfere with the hormonal regulation of cardiovascular system, which may, in turn, relate to the cardiovascular toxicity of this metal.

5.5.4 Effects of Lead on Vascular Reactivity

Addition of Pb acetate to the bathing medium has been shown to elicit a cumulative concentration-dependent vasoconstriction in isolated rabbit mesenteric artery (Watts et al., 1995). This effect was reported to be partly mediated by activation of PKC. In a more recent study, Valencia et al. (2001) found a concentration-dependent vasoconstrictive response to Pb acetate (0.1 to 3.1 mM) in Wistar rat thoracic aorta rings. The contractile response was observed in both intact and endothelium-denuded rings. Likewise, Pb-induced vasoconstriction was preserved in calcium-free medium and was unaffected by either α-1 blockade (prazosin), PKC inhibition (Calphostin) or L-type calcium channel blockade (verapamil). However, Pb-induced vasoconstriction was inhibited by lanthanum, which is a general calcium-channel blocker. These observations suggest that Pb can promote an endothelium-independent vasoconstriction by a direct effect on the vascular smooth muscle cells. The data further suggest that the effect of Pb is Ca-independent and may depend on the entry of Pb to the cell via a lanthanum-blockable channel. In contrast to the latter studies, addition of Pb acetate did not cause vasoconstriction in the rat aorta rings used in a study reported by Shelkovnikov and Gonick (2001). Moreover, Pb acetate at either high (10^{-4}m) or low (10^{-8}m) concentrations did not modify the response to NE, phorbol ester, or isoproterenol. However, at 10^{-4}M, Pb acetate augmented the contractile

response to submaximal concentrations of calcium. Thus, the rapid action of Pb on vascular reactivity in vitro seems to vary depending on the type of the vessel used, the Pb concentration employed, and the animal species being studied.

A number of studies have endeavored to discern possible differences in vascular reactivity to various agonists between animals with Pb-induced HTN and control animals. For instance, Purdy et al. (1997) found no significant difference in vasoconstrictive response to NE and phenylephrine or vasodilatory response to acetylcholine or nitroprusside in the aorta rings obtained from Sprague-Dawley rats with Pb-induced HTN. In contrast, Marques et al. (2001) showed a significant reduction of vasodilatory response to both acetylcholine and nitroprusside in Wistar rats with Pb-induced HTN. It should be noted that the Wistar rats employed in the latter study had been treated with 5 ppm Pb acetate in the drinking water for 1 month, whereas those reported by Purdy et al. (1997) had been given a higher dosage (100 ppm) for a longer period (3 months). Therefore, the magnitude and duration of exposure may account for the differences observed between the two reports. Also, the effect of Pb on vascular reactivity may vary from one tissue to the next, as clearly exemplified by studies (Oishi et al., 1996) that showed significant endothelium-dependent vasorelaxation of mesenteric artery response to acetylcholine in the presence of the NOS inhibitor L-NAME in tissues from rats exposed to Pb acetate for 3 months. These observations suggest that chronic Pb exposure may impair endothelium-dependent hyperpolarization in the rat mesenteric artery. However, no such effect was noted in the aorta obtained from the same animals.

5.5.5 Lead-Calcium Interactions in Vascular Tissue

Changes in cytosolic Ca^{2+} concentrations are intimately involved in regulating vascular tone and vascular smooth muscle contraction. Consequently, several studies have focused on the interaction of Pb with cellular Ca^{2+} and Ca^{2+}-dependent signaling pathways as a means to gain insight into the pathogenesis of Pb-induced HTN (Piccini et al., 1977; Favalli et al., 1977; Webb et al., 1981; Goldstein, 1993; Watts et al., 1995). Lead can potentially compete with Ca^{2+} in transport systems (i.e., channels and pumps) involved in physiological movements of ions, particularly Ca^{2+}, into and out of the cell (Simons, 1993a,b). Moreover, Pb can alter the intracellular distribution of Ca^{2+} between cytoplasm, endoplasmic reticulum, and mitochondria, which normally regulates cytosolic Ca^{2+} concentration, (Simons 1993a,b). In addition, Pb can

serve as a substitute for calcium in Ca^{2+}-dependent signaling pathways by interacting with calmodulin, PKC, and calcium-dependent potassium channels (Haberman, 1983; Richardt et al., 1986; Chai and Webb, 1988; Simons, 1993a,b; Watts et al., 1995). Thus, interactions of Pb with cellular Ca^{2+} via these complex mechanisms in the vascular cells may contribute to alterations of vascular resistance and HTN. For example, Piccini et al. (1977) and Favalli et al. (1977) showed that Pb exposure increases calcium content in the tail artery in rats. The authors attributed this phenomenon to a possible Pb-induced inhibition of Ca^{2+} extrusion from the vascular cells. Using rabbit mesenteric artery preparations, Watts et al. (1995), showed that blockade of either PKC or voltage-gated Ca channels by verapamil substantially attenuated Pb-induced vasoconstriction in both intact and endothelium-denuded preparations. Based on these observations, the authors suggested that Pb promotes a vasoconstrictive response in rabbit mesenteric artery via a Ca^{2+}-dependent activation of PKC. In contrast, Valencia et al. (2001) using rat aorta rings reported a vasoconstrictive response to Pb acetate in rat aorta rings bathed in either Ca^{2+}-free or Ca^{2+}-containing media and in the presence or absence of the L-type calcium-channel blocker verapamil or of the PKC inhibitor calphostin. Moreover, depletion of intracellular Ca^{2+} stores by preincubation of rings in EGTA, while diminishing the intensity, did not abrogate Pb-induced vasoconstriction in this system. In contrast, Pb-induced vasoconstriction was prevented by lanthanum (a general blocker of calcium channels) in both Ca^{2+}-containing and Ca^{2+}-free media. Based on these observations, the authors concluded that Pb can elicit a PKC-independent contractile response in the rat aorta by entering VSMC via a non-voltage-gated Ca^{2+} channel and mimicking the action of Ca^{2+}. It, thus, appears that Pb exerts its effect by mechanisms that are species- and vessel-specific.

5.5.6 Cardiotoxicity and Atherogenesis

Acute Pb exposure has been reported to affect cardiac function, and chronic exposure has been linked to atherosclerosis and increased cardiovascular mortality by some, but not by all investigators, in humans (See Chapter 6). In an attempt to assess the cardiotoxicity of Pb, Prentice and Kopp (1985) carried out the in vitro perfusion of isolated rat heart preparations with a perfusate containing 0.3 and 30 μM Pb acetate for up to 60 min. At 30 μM concentration, Pb prolonged the AV node and His bundle conduction times, reduced coronary blood flow and heart rate, and altered cardiac energy metabolism. Milder, and statistically insignificant, changes were

also observed at 0.3 μM Pb concentration in this model. These observations illustrate the direct cardiotoxicity of Pb independently of its systemic and neuroendocrine actions in acute intoxication. In an attempt to determine whether chronic exposure to Pb or cadmium can cause atherosclerosis, Revis et al. (1981), studied male white pigeons that were exposed to Pb (0.8 ppm in drinking water) for extended periods. Long-term low-level Pb exposure in this model resulted in a significant rise in arterial pressure and a near doubling of the number of atheromatous plaques in the aorta. These observations demonstrate the proatherogenic effects of chronic exposure to low levels of Pb in pigeons.

5.5.7 Effects of Lead on Endothelial Cells

Endothelium is an important constituent of the blood vessel wall, which regulates macromolecular permeability, vascular smooth muscle tone, tissue perfusion, and blood fluidity. Endothelial damage or dysfunction results in atherosclerosis, thrombosis, and tissue injury. Chronic Pb exposure has been shown to promote atherosclerosis in experimental animals (Revis et al., 1981). Given the central role of endothelial injury/dysfunction in the pathogenesis of atherosclerosis, numerous studies have explored the effect of Pb on cultured endothelial cells. These studies have searched for evidence of Pb-mediated endothelial cell injury and the effects of Pb on endothelial cell proliferation, tube formation (angiogenesis), monolayer wound repair, and production of heparansulfate proteoglycans, plasminogen activator (tPA), and plasminogen activator inhibitor-1 (PAI-1).

Using cultured bovine aorta endothelial cells, Kaji et al. (1995a) showed that incubation with Pb nitrate at concentrations equal to or below 50 μM for 24 h, results in mild de-endothelialization of endothelial monolayers in vitro. They further showed that adding Pb at 10 μM concentration markedly increased cadmium-induced endothelial injury.

Proliferation of endothelial cells is a critical step for the repair of injured endothelium. Failure of the repair process can result in thrombosis, VSM cell migration and proliferation, and atherosclerosis. In this regard, Pb (Pb nitrate 0.5 to 5 μM) has been shown to significantly reduce DNA synthesis and cell proliferation in growing cultured bovine aorta endothelial cells (Kaji, 1995a). Similarly, the proliferative response to βFGF and αFGF is significantly attenuated by Pb in this system (Kaji, 1995b). The reported inhibition of endothelial cell proliferation by Pb can potentially diminish the repair process in response to endothelial injury. This supposition

has been confirmed by Fujiwara et al. (1998) who showed that at 5 to 10 μM concentrations, Pb markedly inhibited the repair of the wounded endothelial monolayer in vitro. Moreover, Pb severely mitigated the zinc-stimulated endothelial cell proliferation and repopulation of the denuded sections in this system.

Endothelial cell proliferation is the primary step in angiogenesis, a phenomenon that is essential for numerous physiological functions such as growth, development, wound repair, and menstrual cycle as well as certain pathological events including diabetic retinopathy and tumor growth. In view of the demonstrated inhibition of endothelial cell growth by Pb, it has been postulated that Pb may impair angiogenesis. This assumption has been confirmed by a number of studies testing the effect of Pb by angiogenesis assay (tube formation) in endothelial cells cultured on matrigel (a laminin-rich basement membrane product) matrix in vitro. For instance, Ueda et al. (1997) and Kishimoto et al. (1995) have shown that Pb acetate (1 to 100 μM) results in a concentration- and time-dependent inhibition of tube formation by human umbilical vein endothelial cells cultured on a matrigel matrix.

Endothelial cell migration and proliferation are critical for angiogenesis and repair of the damaged endothelium. βFGF is a powerful mitogen for endothelial cells as well as several other cell types. Endothelial cells synthesize βFGF, which is released following injury or spontaneous death of endothelial cells and acts in an autocrine fashion to facilitate the repair process by promoting endothelial cell migration and proliferation. Binding of βFGF to its receptor on the endothelial cell is facilitated by heparan sulfate proteoglycans (HSPGs) that are normally produced and released by the endothelial cells for attachment to the cell surface as well as incorporation in the extracellular matrix. As noted above, Pb significantly attenuates βFGF and αFGF-mediated DNA synthesis and proliferation in cultured endothelial cells (Kaji et al., 1995b). In this regard, Pb has been shown to reduce βFGF binding to the cell surface HSPGs without changing the biosynthesis or intracellular abundance of βFGF in cultured bovine endothelial cells (Fujiwara and Kaji, 1999). Moreover, Pb has been shown to significantly reduce the synthesis of glycosamino-glycans (GAG, measured by sulfate incorporation into heparan sulfate) in the growing endothelial cells.

The above observations suggest that Pb-induced reduction of βFGF-mediated proliferative response in cultured endothelial cells is largely due to impaired production of HSPGs. This supposition is further supported by observations that DNA synthesis can be restored by adding

heparin in Pb-treated growing endothelial cells (Fujiwara et al., 1995). The reduction in the production of GAGs by Pb in the growing endothelial cells (Fujiwara et al., 1995) is also seen in confluent (quiescent) cells. For instance, Kaji et al. (1991) demonstrated a marked reduction of GAG production following incubation with 10 μM Pb nitrate in confluent endothelial cells in vitro. The Pb-induced reduction of heparan sulfate production was more severe than that of the other GAGs. Moreover, the reduction in the cell surface-associated GAGs was more severe than that of the newly synthesized GAG found in the incubation media. GAGs combine with a series of specific core proteins to form anionic macromolecular complexes known as proteoglycans, which are widely distributed in the extracellular matrix of the mammalian tissues. Endothelial cells produce two types of HSPGs, i.e., the high-molecular weight and low-molecular weight classes. Perlecan is a high-molecular weight heparan-sulfate proteoglycan that is a component of the basement membrane. Syndecan, glypican, ryudocan, and fibroglygan are among the low-molecular weight subclass and are primarily associated with the cell surface. Proteoglycans play an important role in regulating vascular function and structure. For instance, by providing a negative electrostatic charge, these molecules constitute a major barrier against extravasations of negatively charged plasma proteins. In addition, by interacting with antihthrombin-III and tPA, these molecules serve as important endogenous anticoagulants. Moreover, perlecans facilitate βFGF binding to its receptor on endothelial cells and, thus, contributes to the endothelial growth and repair processes. In contrast, these molecules tend to inhibit migration and growth of vascular smooth muscle cells and, thereby, help to prevent athero- and arteriosclerosis. Another important function of HSPGs is their role in stabilizing and anchoring lipoprotein lipase and VLDL receptors on the endothelial surface. Consequently, they play an important indirect part in the clearance of VLDL and chylomicrons from the circulation, a process that has major implications for energy metabolism and cardiovascular protection.

In a study of cultured bovine endothelial cells, Kaji et al. (1997) found that Pb chloride, at 10 μM concentration, markedly lowers incorporation of precursors (glycosamine and sulfate) into HSPG in confluent bovine aorta endothelial cells. The effect of Pb was more severe on low-molecular than high-molecular weight HSPGs. However, Pb did not change the length of heparan sulfate chains. It is of note that Pb slightly increased the abundance of the HSPG core proteins. This observation excluded a reduction in core protein synthesis as a cause of diminished HSPGs in the Pb-treated confluent endothelial cells. In a subsequent study, Fujiwara

and Kaji (1999) investigated the effect of Pb nitrate on production of high- and low-molecular weight subclasses of HSPGs in growing bovine aorta endothelial cells. In contrast to the quiescent cells, Pb-treated growing cells exhibited a marked reduction in the high-molecular weight with no change in production of low molecular weight (~50KD) HSPGs. They further showed a significant reduction of the core protein of perlecan, which is a high-molecular weight (400 KD) HSPG. Thus, Pb appears to affect productions of subclasses of HSPGs differently depending on the cells' growth cycle. Accordingly, in the growing endothelial cells (a condition that simulates the response to injury), Pb downregulates perlecan, which is involved in βFGF-mediated migration and proliferation of endothelial cells and inhibition of migration and proliferation of VSMC. This phenomenon may adversely affect endothelial repair and promote athero- and arteriosclerosis. On the other hand, Pb-induced reduction of the cell surface-associated low-molecular weight HSPGs (which are predominantly involved with lipolytic, anticoagulant, and other functions of confluent endothelial cells (simulating intact endothelium) can contribute to hyperlipidemia and thromboembolism, among other disorders.

One of the major properties of normal endothelium is its ability to prevent coagulation. Several factors contribute to the thromboresistance of the endothelial lining. These include the surface coating of HSPG (which confers heparin-like properties), nitric oxide (which inhibits platelet adhesion and activation), and tPA (which promotes thrombolysis), thrombomodulin, and prostacycline. As noted earlier, Pb exposure reduces HSPG-production (Kaji et al., 1995b, 1997) and diminishes nitric oxide availability via ROS-mediated NO inactivation (Vaziri et al., 1999b). In addition, Kaji et al. (1992) showed that incubation of confluent human umbilical vein endothelial cells with Pb nitrate, at 0.01 to 1.0 μM concentrations, significantly reduced basal and thrombin-stimulated tPA release. It thus, appears that Pb exposure may confer a thrombophilic diathesis.

5.5.8 Effects of Lead on Vascular Smooth Muscle Cells

Lead has been demonstrated to stimulate proliferation of bovine aorta VSMCs in a concentration-dependent manner (Fujiwara et al., 1995). Moreover, the combination of Pb and βFGF results in an additive effect on VSMC proliferation. As with bovine aorta VSMCs, cultured rat aorta VSMCs exhibit hyperplasia in response to a low concentration of (100 μg/L) of Pb-citrate (Carsia et al., 1995). The reported hyperplasia is accompanied by phenotypical

transformation of cells from the spindle or ribbon shape to cobblestone shape, simulating the neointimal cell morphology. This was accompanied by a significant reduction in Ang-II receptor but no change in α, β, or ANP receptor densities. It is of note that, in contrast to the low concentration, a high concentration (500 μM/L) of Pb resulted in growth arrest in this system. Thus, the effect of low concentration of Pb on VSMC proliferation is opposite of its action on the endothelial cells.

Under normal conditions, intact endothelial lining shields the cells residing in the subendothelial tissue, i.e., fibroblasts and VSMCs, from coming into contact with the circulating blood. However, this barrier is lost when the endothelium is injured, an event which can lead to platelet adhesion and fibrin thrombosis formation. Propagation of fibrin thrombus is limited by activation of the fibrinolytic system, which, in turn, depends on the balance between tPA and plasminogen activator inhibitor-1 (PAI-1). In addition to endothelial cells, VSMCs and fibroblasts express tPA and PAI-1. Using cultured human aorta VSMCs and fetal lung fibroblasts, Yamamoto et al. (1997) investigated the effect of Pb chloride on the release of tPA and PAI-1 in vitro. The authors found that Pb causes a significant inhibition of tPA release and a significant increase in PAI-1 release in cultured fibroblasts in a dose-dependent manner. The Pb-treated VSMC exhibited a significant dose-dependent decline in tPA release and to a lesser extent of PAI-1 release. Taken together, exposure to Pb appears to evoke a negative effect on fibrinolytic process by the cellular constituents of the subendothelial tissue.

5.5.9 Summary

- In vivo and in vitro studies published during the past 15 years have considerably expanded our knowledge of the effects of Pb exposure on the cardiovascular system. However, many questions remain unanswered and await further investigation.
- A number of in vivo and in vitro studies conducted during the review period have provided compelling evidence for the role of oxidative stress in the pathogenesis of Pb-induced HTN. Moreover, the effect of oxidative stress on blood pressure has been shown to be, in part, mediated by avid inactivation of NO and downregulation of sGC. In addition, a limited number of in vitro studies have provided indirect evidence that, via activations of PKC and NFκB, Pb may raise vascular tone and promote inflammation.
- Based on several studies that evaluated the role of adrenergic system on Pb-toxicity, chronic low-level Pb exposure appears to increase central sympathetic activity, reduce cardiac and vascular and raise kidney β adrenergic receptor density. These events can, in turn, increase peripheral vascular resistance and renal renin release/production and, thereby, arterial pressure. Since sympathetic outflow is inhibited by NO, inactivation of

NO by oxidative stress may be, in part, responsible for the increased sympathetic activity in Pb-exposed animals.

- The renin-angiotensin-aldosterone system (RAAS) plays an important role in regulating blood pressure and cardiovascular function and structure. The available new data suggest that Pb exposure can raise plasma ACE and kininase activities at different points in the course of Pb-induced HTN in experimental animals. This can, in turn, contribute to the genesis and/or maintenance of HTN. Since renin release (which is responsible for production of ACE substrate, i.e., Ang-1) is, in part, driven by β adrenergic activation, upregulation of renal β adrenergic activity may, in part, account for increased RAAS activity in the Pb-exposed animals.
- The balance in production of vasodilator and vasoconstrictor prostaglandins plays an important role in regulation of blood pressure and cardiovascular function. Studies of the Pb exposed humans have revealed an imbalance in production of prostaglandins favoring a rise in arterial pressure. However, the animal and in vitro studies published during the review period have been limited and inconsistent. Further studies are needed to address this issue.
- Based on the available studies, Pb exposure appears to increase endothelin production in experimental animals. This phenomenon can, in part, contribute to the rise in blood pressure in the Pb-exposed animals. For instance, Pb has been shown to cause vasoconstriction and to attenuate acetylcholine- and NO-mediated vasodilatation in some, but not all vascular tissues and in some, but not all, studies. These effects have been variably attributed to Pb-mediated activation of PKC and Ca^{2+}-mimetic action of Pb, among other possibilities.
- Finally, a number of studies have explored the effects of endothelial and vascular smooth muscle cells to explore the possible atherogenic effect of Pb exposure. In this context, Pb has been found to inhibit proliferation of the growing (non-confluent) endothelial cells (mimicking in vivo response to injury), impair tube formation (angiogenesis), and the repair of wounded endothelial monolayer in vitro. Likewise, Pb exposure was shown to reduce production of HSPGs and tPA by confluent endothelial monolayers, events that may favor thrombosis and hyperlipidemia. Lead exposure has been also shown to promote vascular smooth muscle cell and fibroblast proliferation and phenotypic transformation in ways that seem to favor arteriosclerosis and vascular remodeling.
- Among many questions awaiting clarification, a few are of particular interest. For instance, it is not clear as to why low, but not high, levels of Pb exposure cause HTN in experimental animals. Similarly, it is uncertain as to why HTN occurs long after the onset of Pb exposure in the intact animals, whereas the effects on cultured cells and isolated tissues are manifested within short periods of time.

5.6 GENOTOXIC AND CARCINOGENIC EFFECTS OF LEAD

5.6.1 Introduction

The 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a) and its 1990 Supplement (U.S. Environmental Protection Agency, 1990) concluded that, at relatively high concentrations, Pb may be carcinogenic to laboratory animals, particularly the rat. Cell culture studies were considered to be supportive of these observations, but also indicated that Pb was not particularly potent. Human data were considered to be of concern, but not definitive, and given the animal data, the prudent choice was to consider Pb to be a possible human carcinogen.

This section reviews reports of Pb-induced carcinogenesis and DNA damage published since 1986. More than 200 publications were read and considered and those that reported any effect related to carcinogenesis or genotoxicity that was attributable to Pb are presented below.

This report follows the same format as the previous one (1986) and the explanations for the relative importance of the various types of studies (e.g., epidemiology, animal and cell culture) can be found in the original report and are not repeated here. Carcinogenesis studies are presented first, followed by genotoxicity studies. Each of these sections is further subdivided into human studies (considering adults and then children), animal studies, and then cell culture studies (considering human, mammalian, and then nonmammalian). When appropriate, these sections are followed by a section describing acellular (cell-free) model studies.

There are some differences with this new report. For one, each section is more distinctly broken out. The epidemiology has been reviewed in more detail in Chapter 6 (Section 6.7) in this document and, so, only a brief summary is presented here. Because of more recent concerns about effects on childhood development, this issue was specifically considered in a separate section. Following advances in hypotheses and technology, much more specific sections about the possible epigenetic effects of Pb have also been added.

5.6.2 Carcinogenesis Studies

5.6.2.1 Human Studies

The human carcinogenesis studies are only briefly reviewed in this section; for a more detailed review, see Chapter 6 (Section 6.7) in this document.

Adults

The assessment of the carcinogenicity of Pb through human epidemiological studies remains ambiguous. Several reports state that occupational exposure to Pb increases the risk of lung, kidney, brain, stomach, and liver cancer (Fu and Boffetta, 1995; Kauppinen et al., 1992; Gerhardsson et al., 1995; Ades and Kazantzis, 1988; Wicklund et al., 1988; Steenland et al., 1992; Englyst et al., 2001; Gerhardsson et al., 1986; Anttila et al., 1995, 1996; Cocco et al., 1998; Shukla et al., 1998). However, a full interpretation of the data in these studies is complicated by the fact that the study participants also incurred coexposure to other known carcinogens, such as arsenic, cadmium, and hexavalent chromium. Thus, it is difficult to determine if the excess cancers observed were due to exposure to Pb, one of these other carcinogens, or some combination of the various chemicals. In addition, other reports indicate that occupational or environmental exposure to Pb did not alter cancer risk (Cocco et al., 1996; Fanning, 1988; Jemal et al., 2002). Consequently, a definitive assessment of the carcinogenicity of Pb from human studies cannot be made at this time.

Children

There have been no recent studies of Pb-induced cancers in children. This lack of data is not unexpected and is largely because Pb has not been considered a likely cause of childhood cancers. There have, however, been studies of cancers in children resulting from paternal exposure. Here again, the same confounding problems encountered are as seen in the adult population studies, and it is difficult to draw any definitive conclusions. For example, two studies reported elevated childhood tumors (Wilm's tumor and acute nonlymphocytic leukemia) in children whose fathers worked in Pb-related industries, such as welding, painting, and auto repair (Buckley et al, 1989; Olshan et al., 1990). However, workers in these occupations also experienced coexposure to arsenic, cadmium, and hexavalent chromium, and so the cancers observed cannot be solely linked to Pb exposure. In addition, a report from the printing industry in Norway found no link between paternal exposure and childhood cancers and, perhaps, even found a possible reduction in the incidence of childhood cancers with paternal Pb exposure (Kristensen and Andersen, 1992).

The possible interaction of paternal occupation and childhood cancer is an important area of concern. However, a definitive assessment of paternal exposure to Pb cannot be made at this time and more research is needed.

5.6.2.2 Laboratory Animal Studies

Lead is a well-established animal carcinogen, as noted in the 1986 Lead AQCD. Consequently, limited tumorigenesis studies have been conducted in animal models and the focus has been more on the mechanism of neoplasia (e.g., the roles of calcium and metallothionein) and possible immunomodulatory effects of Pb in the promotion of cancer. These studies are summarized in Table AX5-6.1.

All of the studies exposed animals to Pb acetate except one, which focused on Pb chromate. One study investigated the carcinogenicity of a series of chromate compounds, i.e., Pb chromate and several Pb chromate-based compounds were included as part of the group of chromate compounds. The Pb chromate was administered by implantation into the lung after being embedded within a cholesterol pellet. The authors indicated that in this design, Pb chromate was not carcinogenic, but that 4 of the Pb chromate compounds did induce a very rare tumor in the mice. Thus, there is some ambiguity about the carcinogenicity of Pb chromate in the study, as the statistics calculated an expected tumor level based on any tumor and were not based on the occurrence of this very rare (for rats) tumor. It is likely that had the expected value been adjusted for the rare tumor, a conclusion would have been reached that either Pb chromate was tumorigenic or that the study lacked the power to make any determination. The previous EPA report had concluded that Pb chromate is tumorigenic. Thus, it is difficult to draw a firm conclusion from this study.

The remaining five studies focused on Pb acetate (Schrauzer, 1987; Blakley, 1987; Teraki and Uchiumi, 1990; Bogden et al., 1991; Waalkes et al., 2004). In most studies, this compound was administered in drinking water at concentrations from 0.5 to 4000 ppm, but one study considered effects from a subcutaneous (SC) injection both in mice and in rats. Consistent with the findings in the 1986 Lead AQCD, Pb not only induced renal tumors, but also induced other tumors, although the possible effect on mammary tumors is difficult to interpret, as important study details were omitted, as discussed below. In a surprising development, during one lifetime exposure study, Pb suppressed liver tumors (Waalkes et al., 2004).

The key study in this group of studies was a lifetime exposure study that investigated mice exposed to drinking water concentrations of 1,000 to 4,000 ppm Pb and also considered the role of metallothionein. In wild-type mice, Pb acetate induced a low frequency of renal tumors, but hyperplasia was common and exhibited overexpression of cyclin D1. Lead inclusion bodies were also common. Lead also suppressed liver tumors in this study.

By contrast, in metallothionein-deficient mice, Pb acetate induced a high frequency of kidney tumors and severe inflammation. Both the tumors and the regions of inflammation exhibited cyclin D1 overexpression. Lead also suppressed liver tumors in these animals. In contrast to the wild-type mice, Pb inclusion bodies were not seen in these animals.

Another study focused on the ability of Pb to induce tumors in rats after SC injection of Pb acetate (Teraki and Uchiumi, 1990). Tumors formed at the site of injection, and Pb accumulated in the tumors, indicating that Pb is tumorigenic. However, full interpretation of the data is complicated by the absence of data on control animals and the fact that only a single dose was considered.

Three studies investigated compounds that might reduce or prevent Pb-induced cancers, specifically selenium and calcium compounds (Schrauzer, 1987; Bogden et al., 1991). The first study used a rather complex approach to study the possibly protective effects of selenium (Shrauzer, 1987). In this study, mice were infected with the murine mammary tumor virus, because they are known to develop mammary adenocarcinomas when maintained on a low-selenium diet. The data indicated that Pb can induce tumors in these mice even when they are maintained on a high-selenium diet. However, the data are difficult to interpret and the impact of the study is uncertain, as the methods are incomplete, the data on control animals are not provided, and the experimental results are stated but not presented in tables or figures.

The second study investigated the effect of calcium (Bogden et al., 1991). The main focus of this study appeared to be blood pressure, but tumorigenesis was also considered. It might be anticipated that calcium might reduce Pb tumorigenesis by competing for its binding sites or blocking its uptake. However, in this study, calcium did not affect Pb levels in tissue and actually exacerbated Pb-induced carcinogenesis. The full impact of this study is also difficult to assess, as the calcium-treated animals incurred profound nephrocalcinosis.

The remaining study considered Pb-induced immunosuppression as a possible factor contributing to the tumorigenesis induced by other agents, including viruses or chemicals

(Blakley, 1987). The results indicated that Pb may suppress humoral immunity but not cellular immunity. However, this is the only study of its kind and the results need to be repeated in other settings. In addition, it is difficult to determine if these data are specific to the agents used (e.g., murine lymphocytic leukemia virus) or if they represent a class of agents (e.g., viruses in general).

Overall, the above studies confirm that Pb is an animal carcinogen and extends our understanding of mechanisms involved to include a role for metallothionein. Specifically, the recent data show that metallothionein may participate in Pb inclusion bodies and, thus, serves to prevent or reduce Pb-induced tumorigenesis. Much more work is needed to determine the potential exacerbating or ameliorating roles of calcium and selenium and to determine what role Pb-induced immunomodulation may play in the promotion of tumors.

5.6.2.3 Cell Culture Studies

Carcinogenesis is measured in cell culture systems through studies of neoplastic transformation, where morphologically transformed cells are injected into athymic mice to see if the cells can form a tumor in the host animal. Morphological transformation refers to cells that incur a change in morphology, such as formation of a focus (or foci) of cell growth. In addition, for faster study results and as a screening tool, the ability of cells to grow in agar without a surface to attach to (anchorage independence) is often used as a short-term substitute measure for transformation.

Human Cell Cultures

Since the 1986 Lead AQCD, only four studies have used human cell culture systems to study the carcinogenesis of Pb compounds. One found that Pb acetate induced anchorage independence in primary human foreskin fibroblasts (HFF) (Hwua and Yang, 1998). The full impact of these data is uncertain, as previous studies of known metal carcinogens in primary HFF found that these carcinogens induced anchorage independence, but those anchorage-independent cells ultimately senesced. These studies are summarized in Table AX5-6.2. Further study is needed to confirm that Pb can induce anchorage independence and to see if these cells can progress to full neoplastic transformation.

In an effort to explore the importance of oxidative metabolism in inducing anchorage independence, Hwua and Yang (1998) also co-treated some cells with 3-aminotriazole, a known

catalase inhibitor. This co-treatment had no effect on Pb acetate-induced anchorage independence, suggesting that catalase was not involved in this effect. It would be premature to conclude that oxidative metabolism is not involved in anchorage independence, as these are the only data available and are limited to catalase only. More data are needed to elucidate whether oxidative metabolism is involved in this lead effect.

The remaining three studies focused on Pb chromate (Biedermann and Landolph, 1987, 1990; Sidhu et al., 1991). Two used similar HFF cells and found that Pb chromate-induced anchorage independence (Biedermann and Landolph, 1987, 1990). However, these anchorage-independent cells ultimately underwent senescence, suggesting that anchorage independence may not be a suitable short-term marker for neoplastic transformation in primary HFF. It should be noted that these studies were focused on the chromate component of this compound and the potential contribution of Pb was not investigated or discussed. By contrast, Sidhu et al. (1991) found that Pb chromate did not induce anchorage independence in a human osteosarcoma cell line, while it did induce full neoplastic transformation of these cells and the transformed cells did grow in agar. It should be noted that this study was also focused on the chromate component of this compound and that the potential contribution of Pb was not investigated or discussed.

The 1986 Lead AQCD did not include any studies of transformation in human cells. Given that other chromate compounds have been shown to induce anchorage independence, it seems quite possible that the data from Pb chromate exposures may represent effects from chromate and not from Pb. Thus, the data currently seem to indicate that Pb can induce anchorage independence in human cells, but its ability to induce neoplastic transformation of human cells is uncertain. Further study of different Pb compounds and the full assessment of their neoplastic potential (i.e., including studies of the ability of treated cells to form tumors in experimental animal models) are needed before definitive conclusions can be drawn.

Animal Cell Cultures

The 1986 Lead AQCD presented several studies demonstrating that Pb compounds could induce anchorage independence and morphological and neoplastic transformation in rodent cell culture systems. Since that report, six studies have further considered the ability of Pb

compounds to induce these effects. Three focused on Pb chromate and three on Pb compounds without the confounding factor of chromate; these studies are summarized in Table AX5-6.3.

Four studies considered Pb acetate, Pb chloride, or Pb nitrate in Syrian hamster embryo and C3H10T1/2 mouse embryo cells (Zelikoff et al., 1988; Patierno et al., 1988; Patierno and Landolph, 1989; Elias et al., 1991). Three found that Pb compounds did not induce transformation (Patierno et al., 1988; Patierno and Landolph, 1989; Elias et al., 1991); but the third study (Zelikoff et al., 1988) indicated that Pb was weakly positive, though no statistics were performed to validate this conclusion. Zelikoff et al. (1988) indicated that the observations were repeated several times, but only showed data from one experimental run. It is unclear why the studies were not averaged together, as multiple repeats would likely have provided the power to detect whether the observed weak increase was significant.

Five studies considered Pb chromate, which induced neoplastic and morphological transformation of Syrian hamster and mouse C3H10T1/2 embryo cells, as well as enhancing viral transformation (Patierno et al., 1988; Patierno and Landolph, 1989; Schectman et al., 1986; Elias et al., 1989, 1991). The focus on Pb chromate was based largely on concern about chromate; but these studies found that Pb chromate was more potent than other chromate compounds, suggesting that Pb may enhance or contribute to the carcinogenicity. Indeed, one study found that combining Pb nitrate with soluble chromate was as potent as Pb chromate and greater than soluble chromate alone (Elias et al., 1991).

Thus, all together, these studies suggest that Pb ions alone cannot transform rodent cells; however, they may be co-carcinogenic or promote the carcinogenicity of other compounds. These data are in contrast to findings described in the1986 Lead AQCD that included a positive study. One possible factor may be exposure duration; the study in question indicated that the Pb-transformed cells were exposed for 9 days. The studies discussed here all exposed cells for 7 days or less. Further careful study of a time course of exposure is necessary to determine whether Pb actually induces transformation in cultured rodent cells.

Nonmammalian Cell Cultures

No carcinogenesis studies were located that used nonmammalian cell culture models.

5.6.2.4 Organ-Specific Studies

No organ-specific or organ culture studies concerning Pb carcinogenesis were located.

5.6.2.5 Carcinogenesis Summary

It remains difficult to conclude whether Pb is a human carcinogen. The assessment of the carcinogenicity of Pb through human epidemiological studies remains unclear. By contrast, the studies confirm that Pb is an animal carcinogen and further extend our understanding of the mechanism to include a role for metallothionein. The cell culture data suggest that Pb can induce anchorage independence, but whether it can induce full neoplastic transformation of human cells is uncertain. Both IARC and NTP have recently upgraded Pb to a 2A classification (probable human carcinogen); in keeping with the most recent EPA cancer guidelines, while acknowledging the inadequacy of the human data, Pb would likely be characterized as a probable human carcinogen.

To conclude, animal tumorigenicity studies clearly implicate Pb (primarily tested as Pb acetate) as being carcinogenic, although i.v. administration has been the main route of exposure employed in such studies. Based on neoplastic transformation in animal cell culture studies, Pb has also been implicated as a carcinogen with chromate.

5.6.3 Genotoxicity Studies

The human genotoxicity studies are only briefly reviewed in this section. For a more detailed review, see Chapter 6 (Section 6.7) in this document.

5.6.3.1 Human Studies

Adults

A number of studies investigating the potential genotoxicity of Pb have been conducted in human populations. Endpoints considered include chromosome aberrations, sister chromatid exchanges (SCE), micronuclei formation, DNA strand breaks, and hypoxanthine guanine phosphoribosyl transferase (HPRT) mutations. In general, these studies were much more specific than the carcinogenesis studies, as correlations with blood Pb levels could be made, other confounders could be ruled out, and the endpoints were more short-term.

The chromosome damage studies are ambiguous and contained some methodological flaws. Four studies were positive (Huang et al., 1988; De at al., 1995; Bilban, 1998; Pinto et al., 2000), while two were negative (Anwar and Kamal, 1988; Rajah and Ahuja, 1996). Moreover, the four positive studies included two that could not rule out potential contributions from other genotoxic metals and one that found a correlation only at very high blood Pb levels (>52 µg/dL).

By contrast, the studies of micronucleus formation (Bilban, 1998; Vaglenov et al., 1998; Pinto et al., 2000; Palus et al., 2003; Minozzo et al., 2004), SCE (Huang et al., 1988; Bilban, 1998; Pinto et al., 2000; Duydu et al., 2001; Palus et al., 2003), and DNA strand breaks (Restrepo et al., 2000; Fracasso et al., 2002; Hengstler et al., 2003; Danadevi et al., 2003; Palus et al., 2003) all consistently found clear correlations between Pb and genotoxicity. It should be noted that there were two negative studies for SCE (Rajah and Ahuja, 1995, 1996), but both were by the same group and considered the same very small population of workers (only 5 Pb-exposed workers) and, thus, may not have had enough power to detect potential differences.

It is notable that one study found an interesting correlation of HPRT mutation rates and blood Pb levels from environmental Pb exposure in Belgian women (Van Larebeke et al., 2004). This study is the first and only one to consider Pb-induced mutations. Further research is needed to assess the validity of these findings.

Thus, it appears from these studies that Pb is genotoxic to humans, although it may not induce substantial amounts of chromosome damage. This conclusion is consistent with the laboratory studies discussed below. For more in-depth consideration of the epidemiology studies, see Chapter 6, Section 6.7.

Children

Two recent studies of Pb-induced genotoxicity in children have been published. One study of children living in a high Pb-contamination area of Czechoslovakia found no increase in chromosome damage in white blood cells compared with children living in an area with lower Pb contamination (Smejkalova, 1990). Comparisons were not done with children living in an area with little or no Pb contamination. Analyses of blood Pb levels indicated a statistical difference in blood levels between the two groups but not necessarily a substantial, or biologically significant, difference between them. (Typically, the control group levels were in the high 20s compared to the low 30s µg/dL in the exposed group). Thus, the possibility that each group was

exposed to a Pb level that could induce a baseline level of damage cannot be ruled out, and it cannot be conclusively stated that Pb was not clastogenic in this study.

The other study found an increase in Pb-induced strand breaks in white blood cells from children living in an area of Mexico with high Pb contamination compared to children living in an area with lower Pb contamination (Yánez et al., 2003). Blood Pb levels confirmed a difference in exposure to Pb, but urinary arsenic levels also showed that these children were exposed to higher levels of arsenic, too; and, thus, it cannot be determined which chemical was responsible for the damage.

The possible genotoxicity of Pb for children is an important concern. However, there are simply too few data to draw definitive conclusions, and more research is needed. See Chapter 6 (Section 6.7) for more in-depth discussion of the epidemiology of Pb in human populations.

5.6.3.2 Laboratory Animal Studies

Fourteen studies evaluated the genotoxicity of Pb compounds in animal models. The majority of these studies focused on mice, and the Pb was administered by intraperitoneal (IP) or intravenous (IV) injection. Several endpoints were considered, including SCE, chromosome aberrations, micronucleus formation, and DNA strand breaks. Overall, the results are ambiguous, due in part to study design and the various endpoints considered. These studies are summarized in Table AX5-6.4.

Lead compounds appear to be able to damage chromosomes, if only weakly. Two studies with well-performed analyses were positive (Fahmy, 1999; Aboul-Ela, 2002). The other positive studies observed that Pb could induce karyotypic arrangements, indicating a possible clastogenic response; however, these studies did not analyze very many cells (Chakraborty et al., 1987; Nayak et al., 1989a,b; Dhir et al., 1990, 1992a,b; Nehez et al., 2000). Some found chromosome damage, but it did not increase with dose (Chakraborty et al., 1987; Nayak et al., 1989a,b; Dhir et al., 1990). Altogether, the data suggest some role for Pb in inducing chromosome damage, but it may be a weak effect.

Similarly, the data for micronuclei and DNA damage are ambiguous. One study found that Pb induced micronucleus formation in a dose-associated manner, but only considered two doses (Roy et al., 1992). The other study found that Pb induced micronucleus formation but not

in a dose-dependent manner (Jagetia and Aruna, 1998). This difference may reflect the somewhat shorter exposure time in the second study.

One DNA damage study found that Pb nitrate could induce DNA strand breaks in the white blood cells of mice (Devi et al., 2000); however, the damage was not dose-dependent. Another found DNA damage in a number of organs, but only one dose was considered and the authors described the effect as weak (Valverde et al., 2002). In both studies, the highest doses caused less damage than the moderate to low doses. These data again suggest that Pb is only weakly causing damage.

By contrast, the results for SCE have been consistently positive. The three studies that were positive found that SCEs were induced in a dose-dependent manner (Fahmy, 1999; Nayak et al., 1989a; Dhir et al., 1993).

The route of administration complicates the interpretation of all of these genetic studies. All of the studies, except for three chromosome damage studies, used injection-based exposures. It is unknown if exposures that reflect more realistic scenarios (e.g., Pb exposure via drinking water) would cause any of these effects. Only one study of DNA strand breaks used a physiologically relevant exposure (inhalation).

Four studies exposed animals by gavage, which is still a somewhat artificial exposure. One was a DNA damage study that found weak activity (Devi et al., 2000). The other three considered chromosome damage (Aboul-Ela, 2002; Dhir et al., 1992b; Nehez et al., 2000). Two found a dose-response for a 24 h-exposure to Pb nitrate-induced chromosome aberrations in mice (Aboul-Ela, 2002; Dhir et al., 1992b). The other found that a 4-week exposure to Pb acetate induced aneuploidy, but not chromosome aberrations, in rats (Nehez et al., 2000). It is difficult to reconcile these two studies, as they use different exposure times, chemicals, and species. More work is needed using relevant doses and exposure conditions to Pb compounds in multiple species to determine if Pb induces chromosome aberrations.

Some studies also tried to offset the effects of Pb with a variety of compounds. Potential modulators included fruit extract from *Phyllanthus emblica*, ascorbic acid, calcium, and iron (Aboul-Ela, 2002; Dhir et al., 1990, 1992a, 1993; Roy et al., 1992). Other studies sought to determine if coexposure to other toxicants would potentiate the effects of Pb (Dhir et al., 1992b; Nehez et al., 2000) and considered both zirconium and cypermethrin. The data indicated that the fruit extract could block the toxic effects of Pb, an effect that may, in part, be attributable to

ascorbic acid, but that other components must also be involved, because ascorbic acid alone produced variable results. Iron also had an effect, but only if given just before, or with, the Pb compound; post treatments with iron had no effect. Calcium had a strong effect.

The effects seen with zirconium and cypermethrin are less clear. Both were reported to exacerbate the effects of Pb, but the effects for both are complicated by experimental design problems. For example, zirconium only exacerbated Pb's effects when given simultaneously but not when given 2 h before, or after, Pb. This seems rather unusual, as the total exposure to each was 24 h and, thus, simultaneous exposure occurred in every circumstance. Hence, the data seem to suggest that a 22-h coexposure had no effect, but that a 24-h exposure did. There may have been an interaction of the two chemicals in the gut during coexposure, creating a more toxic species.

Interpretation of the cypermethrin study is complicated by its design and the results. Only 20 metaphases were analyzed per animal, instead of the recommended 100. Also, the statistical analyses were done relative to untreated controls and not to animals treated with Pb or cypermethrin alone. Careful inspection of the tables reveals that actual exposure to Pb plus cypermethrin induced less damage than that induced by Pb alone. Thus, the effects of them together appear to be less than additive. More work is needed to explore the meaning of these data and the importance of Pb mixtures.

The previous report revealed a similar amount of ambiguity; some animal studies were positive for chromosome damage and others were negative. Other endpoints were not described after Pb exposure in experimental animals. These data suggest that Pb can induce SCE, but that it can induce chromosome damage, DNA damage, or micronuclei either weakly or not at all.

5.6.3.3 Cell Culture Studies

Few cell culture studies were reported in the 1986 Lead AQCD. Since 1986, a great deal of theoretical and technological progress has allowed for a large number of cell culture studies to be performed, as discussed below.

Human Cell Culture

Mutagenicity

Two studies considered Pb acetate-induced mutagenesis in human cells. Both considered mutations at the HPRT locus, with one using keratinocytes and the other skin fibroblasts (Ye, 1993; Hwua and Yang, 1998). These studies are summarized in Table AX5-6.5.

One study reported no Pb-induced mutagenesis (Hwua and Yang, 1998) but sought to explore the importance of oxidative metabolism in Pb-induced mutagenesis by co-treatment with 3-aminotriazole, a known catalase inhibitor. This co-treatment did not increase Pb acetate-induced mutagenesis, suggesting that either catalase was not involved in this effect or that Pb is truly not mutagenic. It would be premature to conclude that oxidative metabolism is not involved in anchorage independence, as these are the only data and are limited to catalase. More data are needed to elucidate whether oxidative metabolism is involved in this effect of Pb, as well as further studies of Pb-induced mutagenesis.

The other study reported that Pb acetate-induced mutagenesis (Ye, 1993). However, interpretation of this study is hampered by its methodology. The study did not actually measure HPRT mutations or colony formation, but rather it attempted a quicker methodology that measured tritium incorporation. Although a shorter assay is highly desirable, the study did not verify the observed effects with standard methods, and, thus, it is uncertain if the tritium incorporation actually reflected Pb-induced mutations.

One study considered Pb chromate and found that it was not mutagenic (Biedermann and Landolph, 1990).

There are insufficient data at this point to conclude whether Pb is mutagenic in human cells or not, but the few data available are largely negative.

Clastogenicity

Ten studies investigated the ability of Pb compounds to induce chromosome damage in cultured human cells. All but one were essentially from the same research group, and all but two considered Pb chromate. All were done using normal, or nearly normal, human cells. These studies are summarized in Table AX5-6.6.

Only two of those studies focused on the clastogenicity of Pb itself (Wise et al., 2004b, 2005); the remainder used Pb compounds but focused on either chromate or radioactive particles as the clastogenic species. These studies found that Pb glutamate was not clastogenic.

All of the Pb chromate studies found that Pb chromate induced chromosome damage in a concentration-dependent manner. However, the effects were either attributed or demonstrated to be caused by chromate ions. Lead ions were produced by Pb chromate, but they were not clastogenic.

There was one study of radioactive Pb (Martins et al., 1993). The focus was on the clastogenic activity of alpha particles, and the identity of the specific Pb salt was not provided. The alpha particles were able to induce chromosome damage.

Overall, the data appear to indicate that Pb does not induce chromosome damage in human cells, although more investigation of different compounds is needed.

DNA Damage

Studies of DNA damage in cultured human cells have considered DNA strand breaks, Pb-DNA adducts, and DNA-protein crosslinks for a variety of Pb compounds. The only clear positive damage induced by Pb was Pb-DNA adducts following Pb chromate exposure, although the authors referred to them as Pb associated with DNA (Singh et al., 1999). It is uncertain if these represent actual adducts or some weaker association. Two studies found no DNA strand breaks induced by Pb (Hartwig et al., 1990; Snyder and Lachmann, 1989), and one study involving several laboratories found no DNA-protein crosslinks after Pb exposure (Costa et al., 1996). The other study found DNA double-strand breaks, but these were attributed to chromate and not Pb (Xie et al., 2005). These studies are summarized in Table AX5-6.7.

One other study was positive (Woźniak and Blasiak, 2003), but the results were unusual and their impact uncertain. Specifically, this study found that Pb acetate induced DNA single-strand breaks but that the amount of damage decreased with concentration, and ultimately the highest concentration had less damage than the control. DNA double-strand breaks were observed, but were lowest at the highest concentration. DNA-protein crosslinks were seen only at the highest concentration, and the authors attempted to explain the decrease in strand breaks with this effect. This explanation may partially correct, but it does not entirely explain the

decreased amount of damage at the middle concentration. These data need to be repeated by an independent group before they can be fully assessed.

Together, these data suggest that Pb likely does not induce DNA damage; however, the data are still too limited to allow any definitive conclusions.

Human Cell Genotoxicity Summary

The cumulative data suggest that Pb is not mutagenic and does not induce chromosome aberrations or DNA damage in cultured human cells. It is interesting to note that Pb-induced SCEs have not been considered in human cells.

5.6.3.4 Animal Cell Cultures

Mutagenicity

The potential mutagenicity of Pb compounds in rodent cells was considered in six studies. In particular, three mutagenesis systems were considered: mutagenesis at the HPRT locus, the gpt locus, and mutations in sodium-potassium ATPase. The results are highly variable and may be specific to the Pb compound considered in each case. In particular, Pb chromate and Pb acetate appear to be nonmutagenic. Lead acetate was positive but only at highly cytotoxic concentrations. By contrast, Pb chloride and Pb sulfate appeared to be mutagenic at relatively nontoxic concentrations. These studies are summarized in Table AX5-6.8. However, insufficient data exist at this point to conclude whether or not Pb is mutagenic in animal cells.

Clastogenicity

Seven studies investigated the ability of Pb compounds to induce chromosome aberrations in cultured mammalian cells (Table AX5-6.9). Four of these studies considered Pb chromate, and further investigation revealed that chromate was responsible for the clastogenic effect (Wise et al., 1992, 1993; Blankenship et al., 1997). Three of these studies considered other Pb compounds (Wise et al., 1994; Lin et al., 1994; Cai and Arenaz, 1998). All but one were negative and that one only found a small response at a single high dose (Wise et al., 1994). Lower doses had no effect. Considered together, the studies indicate that Pb does not induce chromosomal aberrations in cultured mammalian cells.

Only two studies considered Pb-induced micronuclei in cultured mammalian cells. One was negative (Lin et al., 1994) and the other positive (Bonacker et al., 2005).

Four studies considered Pb-induced SCE in cultured mammalian cells. The results were predominately negative (three studies [Hartwig et al, 1990; Lin et al., 1994; Zelikoff et al., 1988]). Interpreting these studies, however, is complicated by the fact that too few metaphase cells (less than 30 per concentration) were analyzed in each study. The one positive study considered 100 metaphases per concentration, making those data more reliable (Cai and Arenaz, 1998).

DNA Damage

Several measures of DNA damage in cultured human cells have been investigated, including DNA single-strand breaks and DNA-protein crosslinks. Most Pb compounds did not induce DNA single-strand breaks. The exception was Pb chromate, which did induce DNA strand breaks, but this effect was likely a result of the chromate ion. These studies are summarized in Table AX5-6.10.

Both Pb chromate and Pb nitrate induced DNA-protein crosslinks in cultured mammalian cells. These data suggest that Pb is genotoxic in this manner; however, it is thought that the Pb chromate-induced DNA-protein crosslinks result from the chromate and that the method used for Pb nitrate is not sufficiently rigorous. Thus, while the data are certainly suggestive, they are insufficient to make any definitive conclusion.

Nonmammalian Cell Cultures

Only one study was located considering Pb in a nonmammalian model (Table AX5-6.11). This study found that Pb chromate was not mutagenic in a bacterial assay. The compound was studied because of its chromate content and, given that it is the lone study, no definitive conclusions can be reached.

5.6.3.5 Cell-Free Studies

No cell-free studies concerning Pb carcinogenesis or genotoxicity were located.

5.6.3.6 Organ-Specific Studies

One study (Valverde et al., 2002) considered organ-specific effects (see Table AX5-6.4). That study found a different pattern of DNA strand breaks in mice after inhalational exposure to Pb acetate. DNA in the brain and lung were damaged the most, kidney and liver next, then nasal epithelia and leukocytes, with no damage in testicle DNA. These data are intriguing, as they suggest organ-specific responses after a physiologically relevant exposure (inhalation). More research is needed, however, to fully assess the impact of these findings. Moreover, while the damage was statistically significant, the authors described the effects as weak.

5.6.3.7 Genotoxicity Section Summary

There is some ambiguity in the genotoxicity results, as some endpoints were positive while most were negative. Consistent with the animal study data, Pb can induce SCE in rodent cells, but it is unknown if it can do so in human cells because this has not been tested. Lead also seems to induce DNA-protein crosslinks in rodent cells.

5.6.4 Genotoxicity as it Pertains to Potential Developmental Effects

The human genotoxicity studies are only briefly reviewed in this section. For a more detailed review, see Chapter 6 (Section 6.7). Only limited animal data and no cell culture studies focused on this issue as a concern. The available data are described below.

Adults

One study was located that considered the effects of Pb on sperm quality and quantity. This study considered Pb, cadmium, and selenium levels in 56 nonsmoking volunteers (Xu et al., 2003). No effects on sperm quality were correlated with Pb exposure up to 10 µg/L. Two other studies were located on the effects of Pb on sperm morphology in animals (Fahmy, 1999; Aboul-Ela, 2002). Both were positive, indicating that Pb may have an effect on sperm. They also found that Pb induced DNA damage in the sperm (See Table AX5-6.4). These studies are summarized in Table AX5-6.12.

Children

No studies were analyzed that considered the genotoxic effects of Pb in children as a developmental hazard. There are two studies that considered the genotoxic effects of Pb in children. They were discussed in Section 5.6.3.1.

Three studies were located on the fetal effects of Pb nitrate on the fetus (Kristensen et al., 1993; Nayak et al., 1989a,b). Lead induced an increase in resorptions and there were hints of possible fetal chromosome damage, but the methods were poorly described and much more work is needed before conclusions can be drawn. These studies are summarized in Table AX5-6.13.

5.6.5 Epigenetic Effects and Mixture Interactions

Lead has been proposed to be a co-mutagen or possibly a promoter. Thus, a number of epigenetic mechanisms have been proposed. Epigenetic effects occur when a compound such as Pb induces changes in cellular processes that do not result from changes in DNA sequence. In other words, Pb has been proposed to alter cells in ways that may change the cell without breaking or mutating DNA. There are three possible mechanisms: (1) alterations of gene expression that can stimulate cells to grow (mitogenesis) and/or can interfere with DNA repair; (this possibility has been investigated in several studies); (2) interaction with other metals; and (3) alteration of oxidative metabolism. Neither of the latter two have been extensively studied.

5.6.5.1 Gene Expression

It has been argued that Pb may induce or co-induce carcinogenesis by altering cellular metabolism or by altering the metabolism of another chemical. Both whole animal and cell culture studies have been conducted to address this question and are described below.

Animal

Animal studies indicate that Pb can induce the expression of some phase I metabolizing enzymes, such as cytochrome P4501A1, and phase II metabolizing enzymes, such as glutathione and glutathione-S-transferase. These studies are summarized in Table AX5-6.14. Thus, it is plausible that through this mechanism, Pb may act as a co-carcinogen by affecting the metabolism of other chemicals or possibly as a direct carcinogen by enhancing endogenously

induced damage. However, no studies have directly shown that such Pb effects are linked to cancer or alter the potency of another chemical; and, thus, it remains only a plausible hypothesis.

Human Cell Culture Studies

A few human cell culture studies have been done, and these generally confirm the animal studies. These studies are summarized in Table AX5-6.15. Lead has been shown to affect the induction of some phase I metabolizing enzymes (such as cytochrome P4501A1) and phase II metabolizing enzymes (such as glutathione and glutathione-S-transferase and NAPDH oxidase). These experiments also indicate that Pb can affect the metabolism of other carcinogenic compounds, although they do not show that the genotoxic or carcinogenic effects change as a result of these effects; and, thus, more work remains to make this more than just a plausible explanation.

Animal Cell Culture Studies

No animal cell culture studies concerning the effects of Pb on the expression of metabolic genes were located.

5.6.5.2 DNA Repair

It has been argued that Pb may induce or co-induce carcinogenesis by altering the repair of DNA lesions induced by another agent. The greatest focus has been on damage induced by ultraviolet (UV) light. Only cell culture and cell-free studies have been conducted to address this question and are described below.

Human

Only one study considered Pb-induced effects on DNA repair in cultured human cells (see Table AX5-6.16). This study found that coexposure to Pb caused persistence of strand breaks induced by UV light. This persistence suggests that Pb interfered with the repair of these lesions, but direct evidence of that interference was not provided. These are the only data in human cells; and, thus, it cannot be determined if Pb inhibits DNA repair in human cells.

Mammalian Cell Culture Models

Two studies considered Pb-induced effects on DNA repair in cultured mammalian cells. These studies are summarized in Table AX5-6.17. Both found that Pb acetate increased UV-induced DNA damage including SCE, mutagenesis, and cytotoxicity. Lead did not affect strand breaks induced by UV. These data suggest that Pb may indeed inhibit repair, although direct interactions with repair proteins were not demonstrated.

Cell Free Systems

One study considered the effects of Pb on DNA repair proteins (McNeill et al., 2004). That study found that Pb can inhibit APE nuclease in cell-free systems.

5.6.5.3 Mitogenesis

It has been argued that Pb may induce or co-induce carcinogenesis by inducing cells to grow when they should not. Both animal and cell culture studies have been conducted to address this question and are described below.

5.6.5.3.1 Animal

Several studies have considered Pb-induced mitogenesis in animal models. These studies are summarized in Table AX5-6.18. These studies found that Pb can stimulate cell growth, but primarily in the liver. One study did consider TNF-α expression in brain cells, but it was not demonstrated whether these effects were mitogenic. The interpretation of many of the studies is complicated by the exposure method (IV injection), which does not reflect human exposure. In general, the data indicate that Pb is mitogenic to the liver.

Human Cell Culture Studies

A number of studies have considered the potential growth-stimulatory effects of Pb in cultured human cells (Table AX5-6.19). These studies all found that Pb did not stimulate cell growth. Thus, mitogenesis is not a likely epigenetic effect for Pb in human cells.

Mammalian Cell Culture Studies

A number of studies have considered the potential growth-stimulatory effects of Pb in cultured mammalian cells other than the kidney. These studies all found that Pb did not stimulate cell growth. Thus, mitogenesis is not a likely epigenetic effect of Pb in human cells. One study found an increased mitotic index; however, it did not consider possible cell cycle arrest (Lin et al., 1994). Indeed, another study found that Pb increased the mitotic index, because it induced M-phase arrest (Wise et al., 2005).

Other

Lead-induced oxidative damage has been investigated as a potential cause of genotoxic or carcinogenic effects. Generally, the results suggest that Pb only produces low levels of reactive oxygen species, but that it may inhibit some enzymes involved in oxidative metabolism (Table AX5-6.20). Thus, Pb may affect oxidative metabolism, but more work is needed to draw meaningful conclusions.

5.6.5.4 Epigenetic Mechanisms Summary

The collective data support the hypothesis that Pb can induce an epigenetic effect. Lead can alter the expression of metabolic genes in cultured cells and may alter DNA repair, although much more study is needed. Lead may also affect oxidative metabolism or interact with other metals, but again more study is needed. By contrast, it is unclear if Pb is mitogenic. It is mitogenic to the liver in animals, but it is not mitogenic in cultured cells. More study is needed to determine if this difference reflects differences between in vivo and cell culture models or if this property is specific to only certain organs, e.g., the liver.

5.6.6 Summary

- Overall, the above studies confirm that Pb is an animal carcinogen and extends our understanding of mechanisms involved to include a role for metallothionein. Specifically, the recent data show that metallothionein may participate in Pb inclusion bodies and, thus, serves to prevent or reduce Pb-induced tumorigenesis.
- Much more work is needed to determine the potential exacerbating or ameliorating roles of calcium and selenium and to determine what role Pb-induced immunomodulation may play in the promotion of tumors.

- All together, these studies suggest that Pb ions alone cannot transform rodent cells; however, they may be co-carcinogenic or promote the carcinogenicity of other compounds. These data are in contrast to findings described in the1986 Lead AQCD that included a positive study. One possible factor may be exposure duration; the study in question indicated that the Pb-transformed cells were exposed for 9 days. The studies discussed here all exposed cells for 7 days or less. Further careful study of a time course of exposure is necessary to determine whether Pb actually induces transformation in cultured rodent cells.

- The previous report found a similar amount of ambiguity; some animal studies were positive for chromosome damage and others were negative. Other endpoints were not described after Pb exposure in experimental animals.

- These data suggest that Pb can induce SCE, but that it can only induce chromosome damage, DNA damage, or micronuclei either weakly or not at all.

- Overall, the data appear to indicate that Pb does not induce chromosome damage in human cells, although more investigation of different compounds is needed.

- Together, these data suggest that Pb likely does not induce DNA damage; however, the data are still too limited to allow any definitive conclusions.

- There is some ambiguity in the genotoxicity results, as some endpoints were positive while most were negative. Consistent with the animal study data, Pb can induce SCE in rodent cells, but it is unknown if it can do so in human cells because this has not been tested. Lead also seems to induce DNA-protein crosslinks in rodent cells.

- The collective data support the hypothesis that Pb can induce an epigenetic effect.

- Lead can alter the expression of metabolic genes in cultured cells and may alter DNA repair, although much more study is needed.

- Lead may also affect oxidative metabolism or interact with other metals, but again more study is needed.

- It is unclear if Pb is mitogenic. It is mitogenic to the liver in animals, but it is not mitogenic in cultured cells. More study is needed to determine if this difference reflects differences between in vivo and cell culture models or if this property is specific to only certain organs, e.g., the liver.

- The overall conclusions have not changed much from the 1986 Lead AQCD. Lead remains an ambiguous carcinogen in humans and a clear carcinogen in animals.

- Cell culture studies support these conclusions, as effects in rodent cells were not seen in human cells.

- Lead does appear to be genotoxic in human epidemiology studies.

- By contrast, the laboratory studies are more ambiguous in both animal and cell culture studies. In these systems, the genotoxicity in culture is limited to SCE and, perhaps, to DNA-protein crosslinks. For other endpoints, it is only weakly active, if at all.
- Lead has not been evaluated sufficiently as a potential genotoxic hazard, but this probably stems from the fact it appears to be weakly genotoxic.
- The available data suggest that Pb can damage sperm and affect fetuses. More work is urgently needed on this topic.
- Cell culture studies do support a possible epigenetic mechanism or co-mutagenic effects.

5.7 LEAD AND THE KIDNEY

5.7.1 Review of Earlier Work

This section summarizes key findings from the 1986 Lead AQCD with regard to Pb effects on the kidney in animals. Human studies published since 1986 are then reviewed in Section 6.4.

Both in vivo and in vitro studies on several different animal species revealed that renal accumulation of Pb is an efficient process that occurs in both proximal and distal portions of the nephron and at both luminal and basolateral membranes (Victery et al., 1979a; Vander et al., 1977). The transmembrane movement of Pb appears to be mediated by an uptake process that is subject to inhibition by several metabolic inhibitors and the acid-base status of the organism. Alkalosis increases Pb entry into tubule cells via both the luminal and basolateral membranes (Victery et al., 1979b).

Goyer et al. (1970a) were principally responsible for defining the role of renal proximal tubular nuclear inclusion bodies in the response to Pb intoxication. In addition to the early reports of nuclear inclusion bodies appearing in the proximal tubule following Pb exposure (Goyer et al., 1970b), biochemical studies on the protein components of isolated rat kidney intranuclear inclusion bodies have shown that the main component has an approximate molecular weight of 27 kDa (Moore et al., 1973) or 32 kDa (Shelton and Egle, 1982) and is rich in glutamate and aspartate. Goyer et al. (1970c) suggested that the intranuclear inclusion body sequesters Pb, to some degree, away from sensitive renal organelles and metabolic pathways. Goyer and Wilson (1975) and Goyer et al. (1978) also showed that single or repeated

administration of $CaNa_2EDTA$ leads to the disruption of the nuclear inclusion bodies and their removal from the nuclei. Rats treated for 24 weeks with both Pb and $CaNa_2EDTA$ had no inclusion bodies, but showed early interstitial nephropathy. As an extension of this study, Cramer et al. (1974) examined renal biopsies from 5 Pb workers with 0.5 to 20 years of exposure. The two workers with normal GFRs, and shortest exposure duration, showed intranuclear inclusion bodies, whereas the remaining three workers had no intranuclear inclusions but showed peritubular fibrosis.

Formation of intranuclear inclusion bodies was a common pathognomic feature for all species examined. In addition, proximal tubular cytomegaly and swollen mitochondria with increased numbers of cytosomes were also observed (Fowler et al., 1980; Spit et al., 1981). The morphological changes were principally localized in the straight (S3) segments of the proximal tubule. Goyer (1968) and Goyer et al. (1968) had demonstrated earlier that, after Pb exposure, mitochondria were not only swollen but also had decreased respiratory control ratios (RCRs) and inhibited state-3 respiration.

Aminoaciduria has been reported in several studies (Studnitz and Haeger-Aronson, 1962; Goyer et al., 1970b; Wapnir et al., 1979). Other studies have reported increased urinary excretion of electrolytes (e.g., sodium, potassium, calcium, water) following Pb administration (Mouw et al., 1978). Victery et al. (1981, 1982a,b, 1983) found that zinc excretion increased following Pb injection.

Wapnir et al. (1979) observed that Pb acetate administration caused a reduction in renal alkaline phosphatase activity and an increase in Mg-ATPase activity, but no significant changes in NaK-ATPase activity. On the other hand, Suketa et al. (1979) found marked a decrease in renal NaK-ATPase activity following a single oral administration of Pb acetate at a dose of 200 mg/kg, but no change in Mg-ATPase.

Renal ALAD was found to be inhibited by Pb in both acute and chronic experiments (Silbergeld et al., 1982). Renal ALAD was similar to control levels when GSH was present but was significantly reduced in the absence of GSH (Gibson and Goldberg, 1970). Accumulation of both ALA and porphobilinogen was also observed in kidney tissue of Pb-treated rabbits, compared to controls. Other studies have not shown a reduction in renal ALAD following Pb exposure (e.g., Fowler et al., 1980). Higher levels of Pb may be required to cause the reduction

in ALAD reported by Silbergeld et al. (1982), and it may possibly involve Pb-binding proteins in the kidney.

5.7.2 Markers of Renal Toxicity

The establishment and validation of new screening tests for nephrotoxic effects have been principally due to the efforts of the Belgian group (Price et al., 1996; Price, 2000; Lauwerys et al., 1992). They proposed that the following battery of tests be used to screen both environmentally exposed and occupationally exposed individuals: (1) measures of glomerular integrity, i.e., urinary high-molecular weight proteins (albumin, IgG, transferrin); (2) measures of tubular absorption and secretion, i.e., low-molecular weight proteins (retinol binding protein, α-1-microglobulin; (3) measures of tubular integrity, i.e., enzymes, lysosomal N-acetyl β-D-glucosaminidase (NAG), brush border alanine aminopeptidase, brush border intestinal alkaline phosphatase, nonspecific alkaline phosphatase, α-glutathione-S-transferase (GST), and brush border antigens (BB50, BBA, HF5); (4) measures of glomerular and distal tubular function, i.e., prostanoids (thromboxane B2, prostaglandin F2 alpha, 6-keto prostaglandin F1alpha); (5) measures of glomerular structural proteins (fibronectin and laminin fragments); and (6) measures of distal tubular function, i.e., Tamm-Horsfall protein and π-GST. Other useful markers include urinary β_2-microglobulin, as a marker of proximal tubular integrity; PGE_2 and PGF_2, distal nephron markers; kallikrein, a marker of the distal tubule; lysozyme, ribonuclease, and γ-glutamyl transferrase, enzymes reflecting proximal tubule integrity; and sialic acid, an extracellular matrix marker (Fels et al., 1994; Pergande et al., 1994; Taylor et al., 1997). One or several of these urinary markers have been used in screening tests for human Pb workers and in animal studies of renal nephrotoxicity, although none has proved to be specific for Pb.

Questions have been raised about the usefulness of urinary NAG as a nephrotoxic marker due to the absence of light or electron microscopic changes in low-dose Pb-treated animals that showed substantial increases in NAG (vide infra) (Khalil-Manesh et al., 1993b). Furthermore, Chia et al. (1994) found that urinary NAG in workers exposed to Pb correlated best with recent blood lead changes, suggesting that the increased urinary NAG activity reflected an acute response to a sharp increase in the renal Pb burden rather than to exocytosis. Questions have also been raised about the value of measuring the vasoconstricting prostariod cytokine thromboxane B2 (TXB_2) and the vasodilating prostanoid 6-keto prostaglandin F1 alpha (PGF1

alpha). Conflicting results have been reported in human Pb-exposed workers. Cardenas et al. (1993) reported an elevation in TXB_2 and a diminution in PGF1 alpha in 41 Pb-exposed workers in contrast to 41 controls. Hotter et al. (1995), on the other hand, reported that both substances were increased in 69 Pb-exposed workers in contrast to 62 controls. Blood Pb levels in the two worker groups were comparable, i.e., 48 µg/dL in the first group and 43 µg/dL in the second. In animal experiments (Gonick et al., 1998), the excretion of both prostanoids was equal in low-Pb (100 ppm)-fed rats as contrasted to normal controls after 3 months, despite an elevation in blood pressure in the Pb-fed rats. Blood Pb in the Pb-fed rats averaged 12.4 µg/dL compared to 1 µg/dL in the controls. Thus, measurements of these prostanoids remain of questionable value.

Attempts to validate nephrotoxic markers were conducted by Pergande et al. (1994), utilizing Pb-exposed workers as contrasted to normal controls. They found that about 30% of the Pb workers showed an increased excretion of α_1-microglobulin, NAG, ribonuclease, and/or Tamm-Horsfall protein, with positive correlations between these tubular indicators and blood Pb concentration.

5.7.3 Biochemical Mechanisms of Lead Toxicity

Nolan and Shaikh (1992) summarized what was known about biochemical mechanisms underlying Pb-induced toxicity at that time. A more detailed description based on recent animal studies follows in the next section.

The initial accumulation of absorbed Pb occurs primarily in the kidneys. This takes place mainly through glomerular filtration and subsequent reabsorption, and, to a small extent, through direct absorption from the blood. Lead may be taken up by the renal tubular epithelial cells from the basolateral side by active transport of the free ion. Smaller amounts can also cotransport with low molecular weight organic anions. The uptake of Pb through the renal brush border does not appear to occur via any specific carriers. Instead, the process may involve binding of Pb to nonspecific surface sites on the brush border membrane, followed by internalization via endocytosis. Acute kidney damage due to Pb manifests primarily in the proximal tubules. The ultrastructural changes observed in acute experimental Pb nephropathy include both specific and nonspecific effects on the proximal tubular epithelium, e.g., dilation of the endoplasmic epithelium, blebbing of the nuclear membrane, enlargement of the autophagosomes, changes in mitochondrial structure, formation of inclusion bodies. Chronic exposure to Pb affects

glomerular filtration, renal clearance, and tubular reabsorption and can lead to renal failure from interstitial nephritis.

Kidneys of chronically exposed individuals often show fewer or no nuclear inclusion bodies compared to kidneys of acutely exposed individuals. The specific ultrastructural changes associated with Pb nephropathy are the formation of cytoplasmic and nuclear Pb inclusion bodies (discussed at greater length in Section 5.11). These inclusion bodies are not limited to the proximal tubular epithelium, and they have also been observed in peritoneum, astrocytes, neuroblastoma cells, lung cells, and osteoclasts upon Pb exposure. The inclusion bodies are roughly spherical and typically consist of an electron-dense core, with a fibrillary network at the periphery. Research has revealed that the formation of the nuclear inclusion bodies is preceded by the synthesis of cytoplasmic inclusion bodies with a very similar structure. A protein unique to these structures is rich in acidic amino acids and has an isoelectric point of 6.3 and a molecular weight of 32 kDa. Two additional proteins with apparent molecular weights of 11.5 kDa and 63 kDa have been identified in kidney extracts. Both of these proteins have a high affinity, but little capacity, for binding Pb. A Pb-binding protein of 12 kDa molecular weight was identified in the supernatant of brain homogenate from Pb-treated rats. A Pb-binding protein of 10 kDa has also been isolated from the erythrocytes of Pb-exposed workers.

Mitochondrial function, in addition to structure, is very sensitive to Pb. Changes include the uncoupling of oxidative phosphorylation, decreased substrate oxidation, and modification of ion transport processes. Other effects of Pb on cellular energetics include chelation of ATP and inhibition of microsomal NaK-ATPase. These changes may account for the proximal tubular dysfunction seen with acute Pb poisoning in children.

A new area of investigation of the mechanism of Pb toxicity was initially proposed by Quinlan et al. (1988) and Hermes-Lima et al. (1991). Both investigators proposed that free radicals, or ROS, stimulated by Pb, may accelerate iron-dependent lipid peroxidation, causing tissue injury. Hermes-Lima et al. (1991) stated further that ALA, which is formed in large amounts in Pb toxicity, may undergo enolization and autoxidation, yielding ROS. Autoxidation of ALA, in the presence or absence of iron complexes, yields superoxide, peroxide, and hydroxyl radicals. Gurer and Ercal (2000), based on several animal studies to be discussed below, have proposed that antioxidant supplementation following Pb exposure may provide a partial remedy by restoring the cell's antioxidant capacity.

5.7.4 Animal Studies

Two excellent review articles have been written about the effects of heavy metals on, and their handling by, the kidney (Barbier et al., 2005) as well as the mechanisms of kidney cell injury from metals (Fowler, 1992). The interested reader is directed to these reviews, although individual effects and mechanisms are discussed below.

5.7.4.1 Lead Toxicokinetics

DeVries et al. (1998) published a model for Pb toxicokinetics to be used in planning treatment. The model is a four-compartment model with first-order kinetics. The four compartments of this model are blood, bone, liver, and kidney. Soft tissues are represented by the kidney and liver compartments. In addition, intake and excretion are included in the model. Excretion of Pb is mainly via the kidneys (70 to 80%), via bile and feces (15%), via nails, hair, and sweat (8%). The blood makes up the central compartment from which Pb is distributed after uptake in the body. The blood compartment contains about 4% of the total Pb body burden and, within this compartment, the Pb is mainly taken up by erythrocytes. The half-life of Pb in blood is about 30 days. From the blood, Pb is distributed relatively quickly to the soft tissues and bone. The distribution constant from blood to bone is much higher than the one from bone to blood, resulting in the accumulation of Pb in bone. The half-life in the soft tissues is about 30 to 40 days. Most of the body burden of Pb can be found in the bone compartment (~94%), where the half-life of Pb is several decades. Because of the vast amount of Pb in bone, a rebound in blood Pb usually occurs after chelation therapy. This model can be compared with a toxicokinetic model developed by Marcus (1985a,b,c) and further explored by Hogan et al. (1998), as discussed in Chapter 4 of this document.

Dieter et al. (1993) examined the effect of the nature of the Pb salt on the oral intake of Pb in male F344 rats. For 30 days, they administered doses of 0, 10, 30, and 100 ppm Pb in the form of soluble Pb oxide, Pb acetate, Pb sulfide, and Pb ore. At 100 ppm of Pb acetate or soluble Pb oxide, the rats developed ~80 µg/dL of blood and ~200 µg/g of bone Pb levels, whereas rats fed Pb sulfide or Pb ore developed ~10 µg/dL of blood Pb and 10 µg/g of bone Pb. In rats fed Pb acetate or soluble Pb oxide, blood Pb progressively increased with increasing dose, whereas measurable levels of Pb in the other two groups were observed only at the highest dose (100 ppm).

5.7.4.2 Pathology, Ultrastructural, and Functional Studies

Two important series of studies contrast the pathological and functional changes in the kidney after prolonged exposure to Pb, with and without chelation therapy (i.e., DMSA or $CaNa_2EDTA$). In the first series of 3 long-term studies, Khalil-Manesh et al. (1992a,b, 1993b) described the effects of Pb acetate on renal function and morphology in male Sprague-Dawley rats fed a low-calcium diet. Lead acetate was used in concentrations of 0.5% (high dose) and 0.01% (low dose) in drinking water for periods from 1 to 12 months, and then Pb-exposed animals were compared to pair-fed controls (12 rats in each group). In all studies, GFR was measured as ^{125}I-iothalamate clearance by a single injection technique. Urinary markers included NAG, GST, and brush border antigens (BB50, HF5, and CG9) and were expressed as units/g creatinine. Blood and urine Pb were measured prior to sacrifice in each group of animals. Wet and dry weights of kidneys were determined, then the kidneys were processed for light, electron, and immunofluorescent microscopy.

In the first study (Khalil-Manesh et al., 1992a), animals treated with continuous high-dose Pb for 12 months reached a maximum blood Pb of 125.4 ± 10.1 µg/dL after 6 months, at which time the dose of Pb was reduced from 0.5% to 0.1%. Blood Pb at the end of 12 months averaged 55 µg/dL. Urine Pb remained above 100 µg/g creatinine at all times, but it was highest at 3 months, averaging 340 µg/g creatinine. In the Pb-treated animals, GFR was increased above controls at 3 months (1.00 ± 0.14 versus 0.83 ± 0.26 mL/min/100 g body wt, $p = 0.05$), then declined after 6 months to 0.78 ± 0.16 versus 0.96 ± 0.08 mL/min/100 g body wt in controls (Figures 5-9 and 5-10).

As indicated by the ratio of kidney dry/wet weight, increased kidney tissue mass was observed during the first 3 months of Pb exposure, but decreased tissue mass was observed by 12 months. With regard to urinary markers, NAG was elevated above control levels at 3, 6, and 9 months of Pb exposure; GST was elevated at 3, 6, and 12 months of Pb exposure; and no significant differences were observed in the brush border antigens. Proximal tubular nuclear inclusion bodies were present at all time periods in Pb-treated animals. Enlargement of proximal tubular cells and nuclei were seen beginning at 3 months. At 6 months, focal tubular atrophy and interstitial fibrosis appeared, increasing in extent up to 12 months. Mitochondrial alterations, consisting of rounding and elongation, appeared by 1 month and were persistent. Glomeruli were normal through 9 months, but, at 12 months, they showed focal and segmental sclerosis.

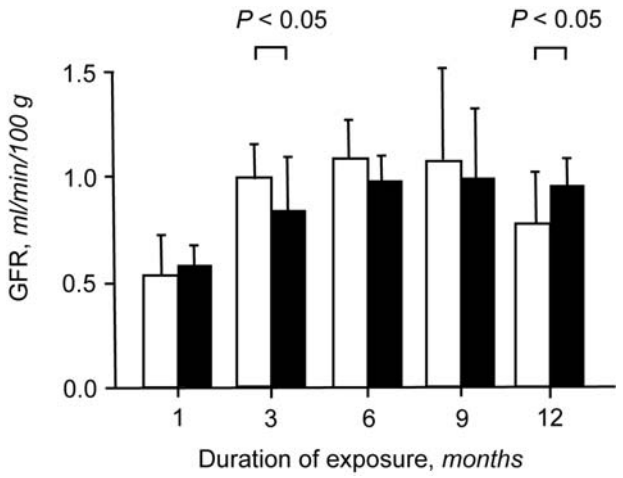

Figure 5-9. Changes in GFR of experimental high-dose lead and control animals with duration of exposure to lead. Open and closed bars represent GFR in experimental and control rats, respectively.

Source: Khalil-Manesh et al. (1992a), with permission.

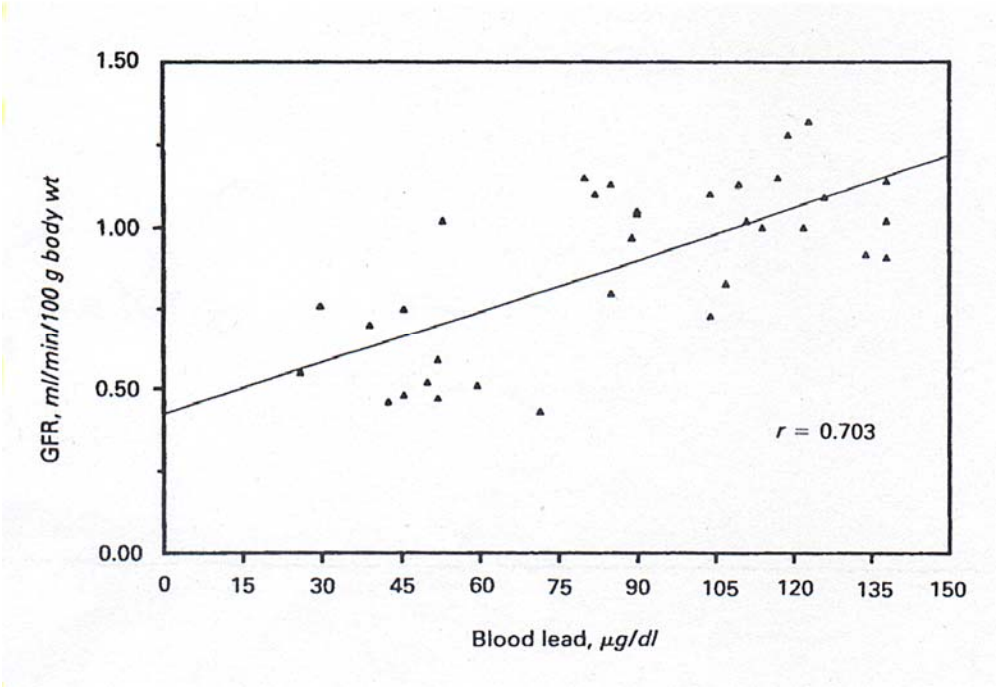

Figure 5-10. Correlation between GFR and blood lead during the first 6 months of high-dose lead exposure.

Source: Khalil-Manesh et al. (1992a), with permission.

There were no electron-dense deposits and immunofluorescent studies were negative. Renal arteries and arterioles were normal at all time point examined.

The second study (Khalil-Manesh et al., 1992b) consisted of the discontinuation of both the high- and low-dose Pb exposure after 6 months, then treatment with three courses of DMSA or discontinuation of high-dose Pb alone after 1, 6, and 9 months of Pb feeding. Controls were pair-fed, exposed to Pb for 6 months, then removed from exposure for 6 months without receiving DMSA. Low-dose Pb-treated rats showed no significant pathologically with or without DMSA treatment but exhibited a significant increase in GFR after DMSA treatment (1.09 ± 0.19 versus 0.88 ± 0.22 mL/min/100 g body weight; $P < 0.03$) (Figure 5-11). Urinary markers remained unchanged, and there were no structural alterations by light or electron microscopy. High-dose Pb-treated animals showed no functional or pathologic changes when Pb exposure was discontinued after 1 month. However, when the duration of exposure was 6 or 9 months, GFR was decreased and serum creatinine and urea nitrogen were increased compared to controls. Tubulointerstitial disease was severe. Administration of DMSA resulted in an improvement in GFR (Figure 5-11) and a decrease in albuminuria, together with a reduction in size and number of nuclear inclusion bodies in proximal tubules.

However, tubulointerstitial scarring was only minimally reduced. In conclusion, except for a brief initial exposure, discontinuation of high-dose Pb exposure failed to reverse Pb-induced renal damage. Treatment with the chelator, DMSA, improved renal function but had less effect on pathologic alterations. Because GFR improved after DMSA treatment in both low- and high-dose Pb-treated animals, irrespective of the degree of pathologic alterations, it may be concluded that the DMSA effect is most likely mediated by hemodynamic changes.

The third study (Khalil-Manesh et al., 1993b) examined the course of events over 12 months in continuous low-level Pb-exposed animals. Maximum blood Pb levels in experimental animals were reached at 3 months, averaging 29.4 ± 4.1 µg/dL. GFR was found to be significantly increased above pair-fed controls at 1 and 3 months, but it was normal at other time periods (1 month experimental, 1.18 ± 0.12 versus control, 0.76 ± 0.15mL/min/100 g; $p < 0.001$; 3 month experimental, 1.12 ± 0.16 , versus control, 0.86 ± 0.10 mL/min/100 g; $p < 0.001$) (Figure 5-12).

Levels of urinary NAG in Pb-exposed rats exceeded control levels at all time periods, except at 12 months, when the normal increase with aging obscured differences between

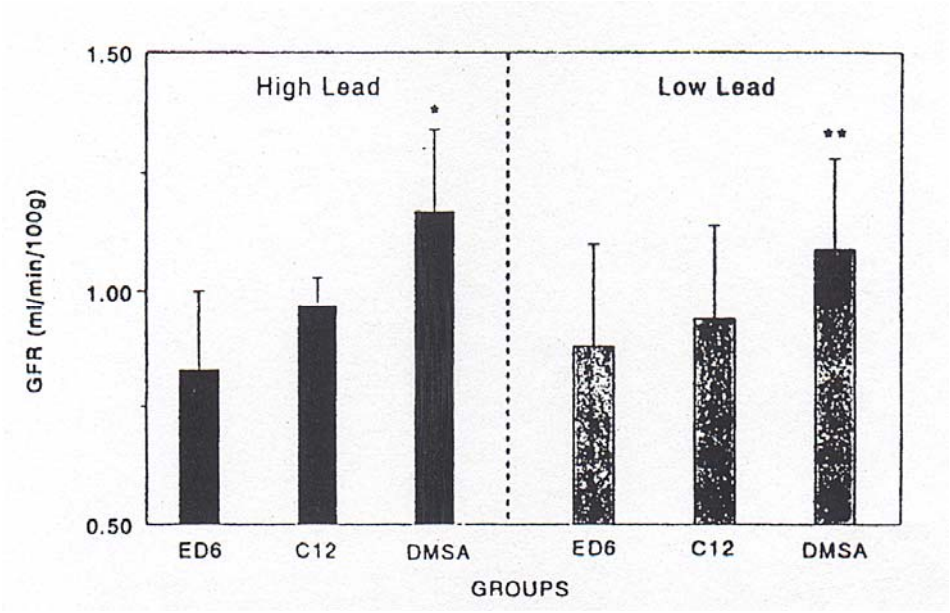

Figure 5-11. GFR in high-lead and low-lead experimental discontinuous (ED6) and DMSA-treated rats (DMSA) as compared to controls (C12). All rats were studied at 12 months.

*p < 0.01 when compared to ED6 and C12.
**p < 0.05 when compared to ED6.

Source: Khalil-Manesh et al. (1992b), with permission.

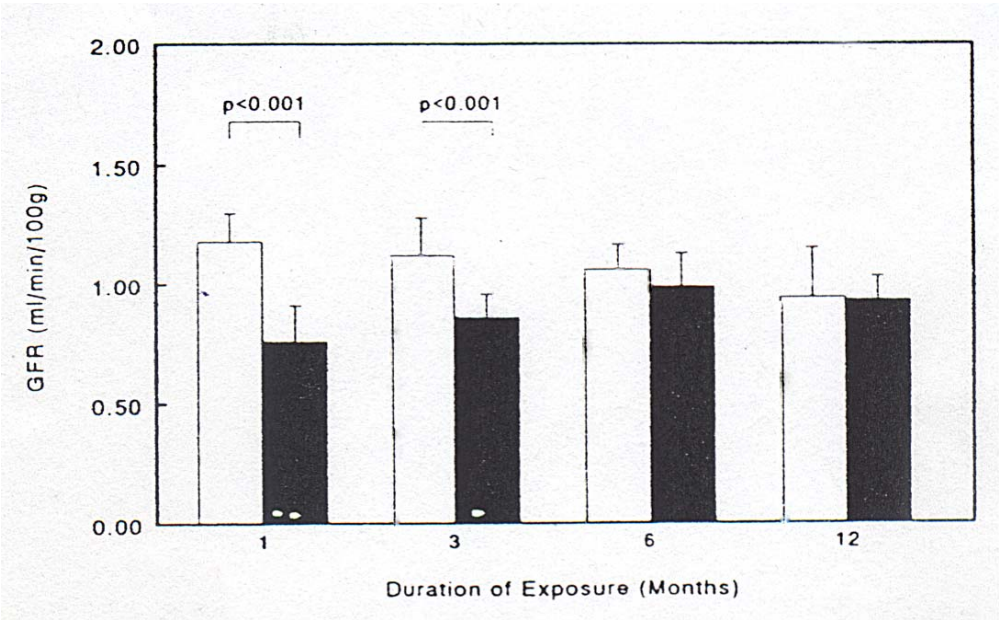

Figure 5-12. Changes in GFR in experimental and control rats, at various time periods.

Source: Khalil-Manesh et al. (1993b).

experimental animals and controls (Figure 5-13). In contrast, urinary GST, a more specific marker of metal-associated proximal tubular injury, was normal at all time periods. Proximal tubular nuclear inclusion bodies were sparse and were observed only at 1 and 3 months.

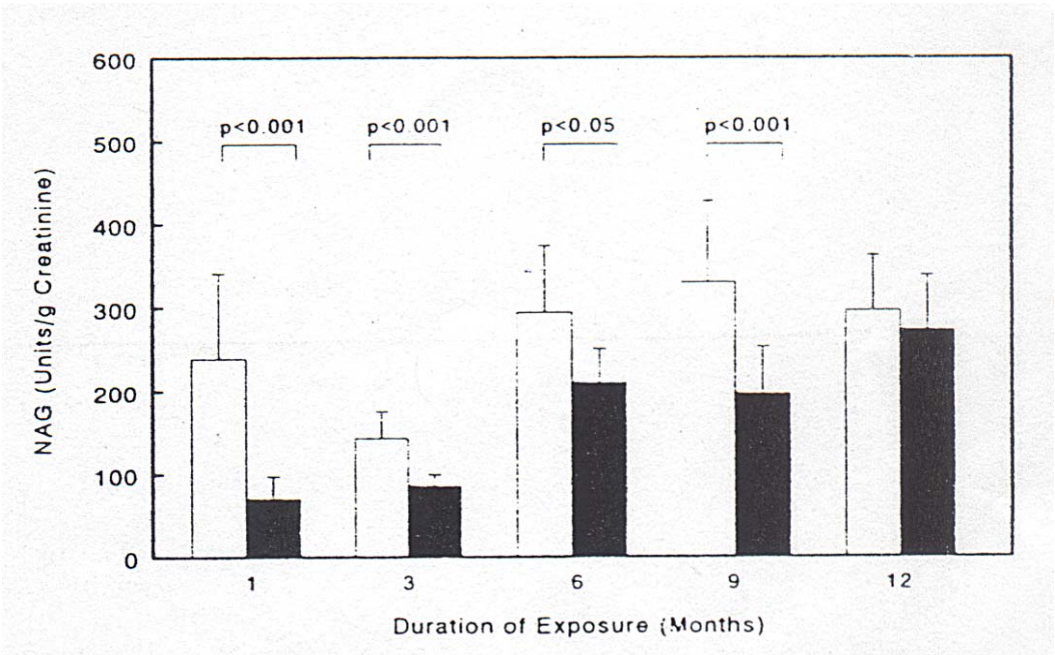

Figure 5-13. Urinary NAG concentration in experimental and control rats at various time periods.

Source: Khalil-Manesh et al. (1993b).

No other pathologic alterations were found in the kidneys until 12 months of exposure, when mild tubular atrophy and interstitial fibrosis were seen. The absence of changes in urinary GST accorded with the relative absence of morphologic changes, whereas the observed increases in urinary NAG suggest that this enzyme may be an overly sensitive indicator of tubular injury, more probably reflecting upregulation of the enzyme even in the absence of tubular injury. It should be noted that both low-dose Pb-treated animals and high-dose Pb-treated animals showed a "hyperfiltration" phenomenon during the first 3 months of Pb exposure. This observation could be invoked as a partial explanation for the late changes of glomerulosclerosis in the high-dose animals, but it cannot explain the lack of glomerular changes in the low-dose animals. Thus, these studies join those of Roels et al. (1994) and Hu (1991) in humans that

indicate that Pb nephropathy should be added to diabetic nephropathy as diseases that lead to early hyperfiltration.

The second series of studies were performed by Sánchez-Fructuoso et al. (2002a,b). Sánchez-Fructuoso et al. (2002a,b) evaluated the effect of $CaNa_2EDTA$ on tissue mobilization of Pb in six-month-old Wistar rats initially treated with 500 ppm Pb acetate for 90 days, followed by treatment with three courses of $CaNa_2EDTA$ 50 mg/kg/day for 5 days, separated by 9 days, or placebo. Lead levels were measured in blood, urine, kidney, liver, brain, and femur. There was no change in bone Pb after $CaNa_2EDTA$ compared to placebo, but Pb levels were significantly reduced in all other tissues (Figure 5-14).

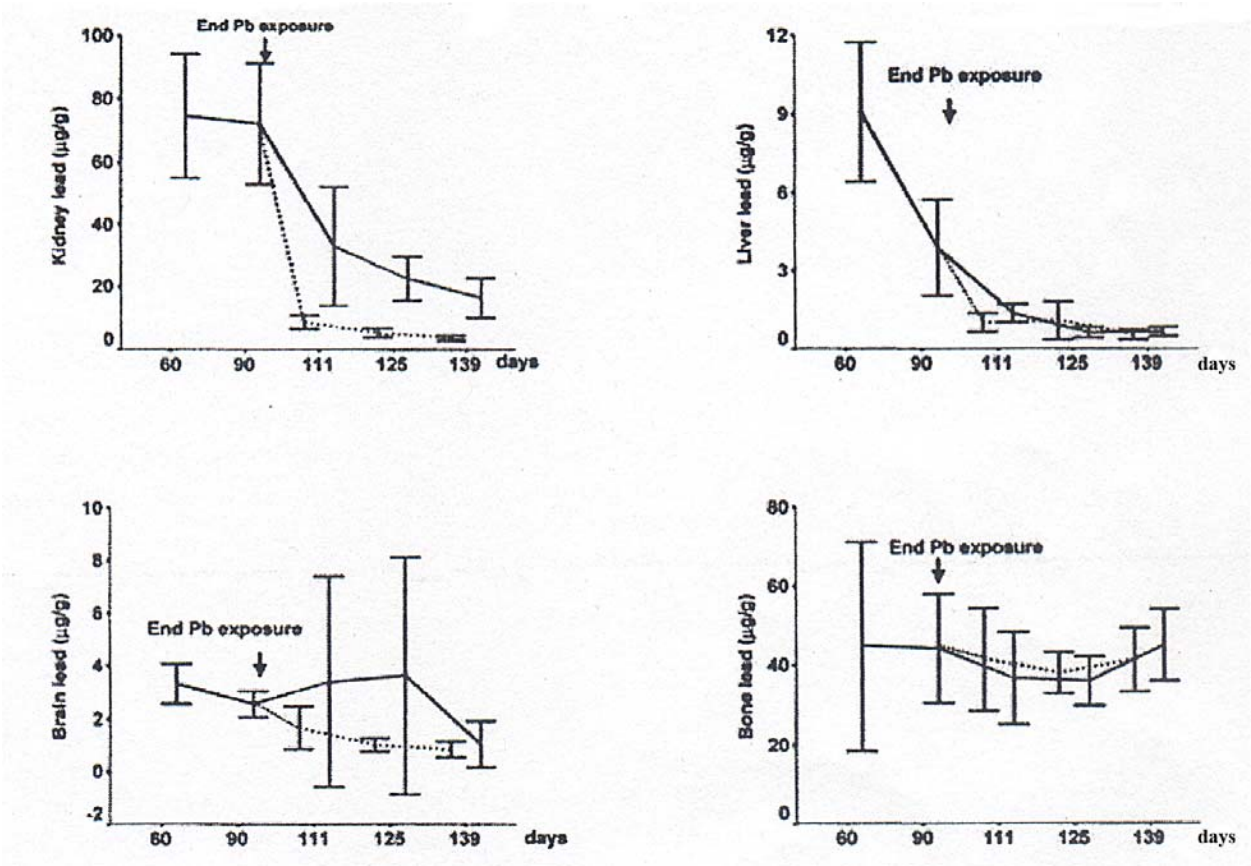

Figure 5-14. Kidney, liver, brain, and bone lead levels in 56 Pb-exposed rats. After 90 days of poisoning, animals were administered serum saline (solid line) or calcium disodium EDTA (broken line).

Source: Sánchez-Fructuoso et al. (2002a), with permission.

The authors emphasized that there was no redistribution to brain. Cory-Slechta et al. (1987) had originally reported that, with one day of $CaNa_2EDTA$ chelation in Pb-exposed rats, Pb is preferentially mobilized from bone and then redistributed to other organs, including brain, but with further $CaNa_2EDTA$ treatment, brain levels return to baseline. The Sánchez-Fructuoso et al. (2002a,b) findings stand in contrast, explained by the authors as being due to a 3-fold higher level of $CaNa_2EDTA$ used by Cory-Slechta et al. (1987).

Sánchez-Fructuoso et al. (2002b) also evaluated pathologic changes, as well as the response of ALAD activity before and after $CaNa_2EDTA$ treatment in the same rats. In the 90-day Pb-treated animals, the main findings were hypertrophy and vacuolization of medium and small arteries (Figure 5-15); mucoid edema and muscular hypertrophy in arterioles; loss of cell brush borders, cell loss, and intranuclear inclusion bodies in the proximal tubule; and fibrosis and the presence of infiltrates in the interstitial component. Treatment with $CaNa_2EDTA$ slowed the progression of most alterations (Figure 5-16) and resulted in a diminution in nuclear inclusion bodies. ALAD activity was reduced from 3.18 ± 0.52 U/mL in controls, to 0.82 ± 0.16 U/mL in the Pb-exposed rats. In the rats treated with $CaNa_2EDTA$, ALAD returned to near control levels (2.98 ± 0.41 U/mL) at 137 days. It is surprising that such remarkable vascular changes were noted in this study, while none were noted in Khalil-Manesh et al. (1992a), even with high-dose Pb for longer periods of time. The kidney content of Pb (mean 74.6 μg/g) was also lower than the mean kidney content at 12 months (294 μg/g) in the Khalil-Manesh et al. (1992a) study. The only explanation for these striking differences that can be offered is that different strains of rats were employed, i.e., Wistar in the Sánchez-Fructuoso (2002b) study and Sprague-Dawley in the Khalil-Manesh et al. (1992a) study. The presence or absence of hypertension cannot be invoked as an explanation, because in another Khalil-Manesh et al. (1993a) study the low-dose Pb animals became hypertensive whereas the high-dose animals did not. These and other related studies are summarized in Table AX5-7.1.

5.7.4.3 Biochemical Mechanisms of Lead Toxicity

Role of Free Radicals (Reactive Oxygen Species)

Since the early 1990s, it has been appreciated that free radicals, now known as reactive oxygen species (ROS), are involved in the manifestations of Pb poisoning, presumably via their adverse effects on tissue integrity and/or their vasoconstrictive effects on vascular endothelium.

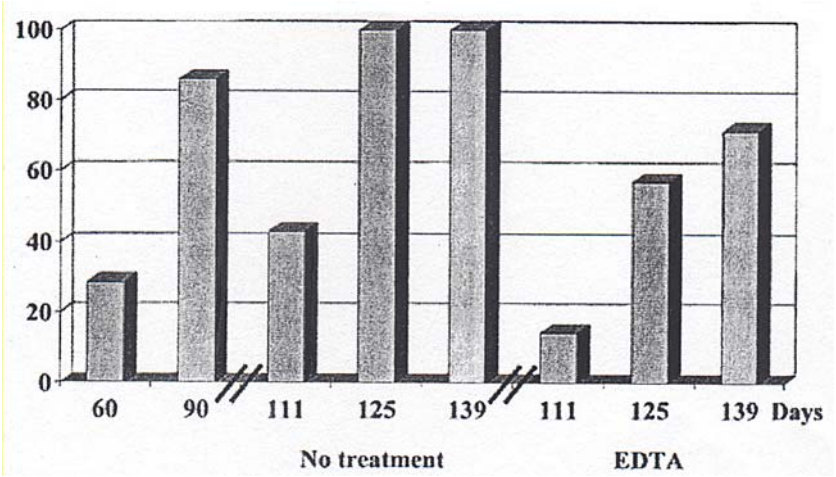

Figure 5-15. Percentage of moderate and severe hypertrophy and vacuolization lesions in small and medium sized arteries in the kidney of lead-exposed rats.

Source: Sánchez-Fructuoso et al. (2002b), with permission.

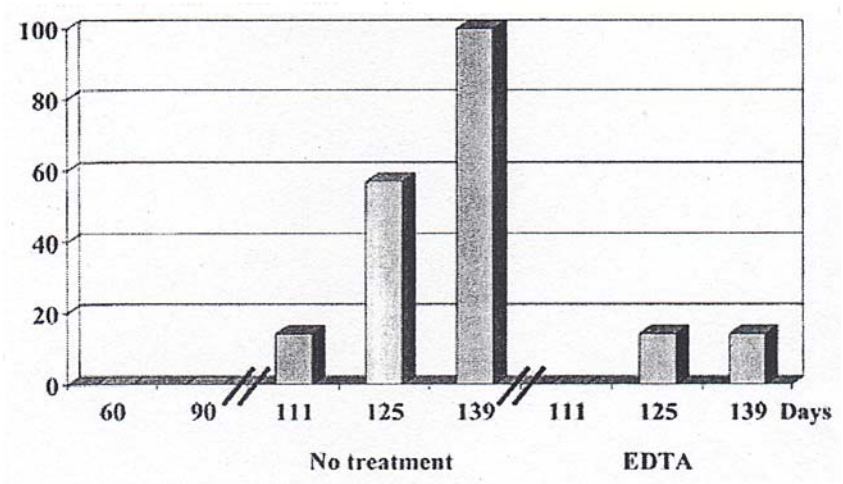

Figure 5-16. Percentage of moderate and severe muscular hypertrophy lesions in arterioles of the kidney in lead-exposed rats.

Source: Sánchez-Fructuoso et al. (2002b), with permission.

Wolin (2000) produced an extensive review of individual ROS, and their interactions with NO, the major endogenous vasodilator, which acts via a second messenger, cGMP. The production of ROS often begins with a one-electron reduction of molecular oxygen to superoxide anion (O_2^-) by various oxidases. NAD(P)H oxidases are the principal enzymes involved.

Superoxide anion is a negatively charged free radical that can be broken down to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) or can interact with NO to form the highly reactive peroxynitrite ion ($ONOO^-$), which, because of its extremely short half-life, is measured as its reaction product, tissue nitrotyrosine. Catalase and glutathione peroxidase (GSHPx) metabolize H_2O_2 to Compound I and oxidized glutathione (GSSG), respectively, while myeloperoxidase metabolizes H_2O_2 to hypochlorous acid (HOCl). The reaction of H_2O_2 with ferrous ion results in the formation of hydroxyl ion ($^{\bullet}OH$). ROS can be scavenged by endogenous thiols (e.g., GSH) or exogenous thiol, e.g., N-acetylcysteine (NAC). ROS can be measured as the concentration of the lipid peroxidation product, malondialdehyde-thiobarbituric acid (MDA-TBA) or by the more recently introduced F-2 isoprostanes.

Kumar and Das (1993) explored the involvement of ROS in the pathobiology of human essential hypertension. They found that plasma levels of lipid peroxides were higher in subjects with uncontrolled essential hypertension compared to normal controls. Angiotensin II, a potent vasoconstrictor, was found to stimulate free radical generation in normal leukocytes, which was thought to inactivate NO, and possibly prostacyclin, which can lead to increased peripheral vascular resistance and hypertension.

Hermes-Lima et al. (1991) also explored the involvement of ROS in Pb poisoning. They described the process of autoxidation of ALA in the presence or absence of iron complexes, which yields free radicals. Free radicals are also produced by Pb-stimulated iron-dependent lipid peroxidation, as determined by quantification of thiobarbituric acid-reactive species (TBARS). Pereira et al. (1992) demonstrated that chronically ALA-treated rats (40 mg/kg body weight every 2 days for 15 days) under swimming training reached fatigue significantly earlier than the control group, as well as demonstrating decreased mitochondrial enzymatic activities. In vivo prooxidant properties of ALA were also suggested by the observed increase of CuZnSOD in brain, muscle, and liver of untrained rats submitted to chronic treatment with ALA.

Ercal et al. (1996) contrasted the effects of treatment with DMSA or NAC in Pb-exposed C57BL/6 mice. Five weeks of Pb exposure was found to deplete GSH levels, increase GSSG, and promote MDA production in both liver and brain samples. Glutathione levels increased and GSSG and MDA levels decreased in groups of Pb-exposed mice that received 1 mmol/kg DMSA or 5.5 mM/kg NAC for 7 days prior to sacrifice. Treatment with DMSA caused reduction in blood, liver, and brain Pb levels consistent with its function as a chelating agent, while treatment

with NAC did not reduce these Pb levels. However, NAC treatment reduced indices of oxidative stress in both brain and liver samples. Blood Pb concentrations in controls were 0.5 ± 0.5 μg/dL; in Pb-treated mice, 36.5 ± 2.4 μg/dL; in Pb + DMSA-treated mice, 13.7 ± 1.3 μg/dL; and in Pb + NAC-treated mice, 36.0 ± 3.5 μg/dL. Thus, both DMSA and NAC acted as antioxidants, presumably via their thiol groups, but only DMSA reduced Pb concentration.

Daggett et al. (1998) and Fowler et al. (2004) have explored the effects of Pb and Pb mixed with cadmium and arsenic on oxidative stress in the rat kidney. Daggett et al. (1998) found that a single injection of Pb failed to deplete GSH or alter MDA levels in the kidney within 24 hours. All subunits of glutathione-S-transferase (GST), however, were increased, apparently not as the result of oxidative stress. Fowler et al. (2004) reported preliminary studies of oxidative stress produced at 30, 90, and 180 days by mixtures of Pb (Pb acetate at 25 ppm), cadmium (cadmium chloride at 10 ppm), and arsenic (sodium arsenite at 5 ppm). These dosages were at the lowest observed adverse effect levels (LOAEL). Kidney carbonyls (a marker of protein oxidation) were increased at all time points in the combination group, but decreased at 90 days in individually administered metal groups. Kidney non-protein thiols (representing glutathione) increased in all groups at 180 days, suggesting that the induction of glutathione or metallothionine attenuated increases in oxidative stress.

Vaziri and co-workers (Gonick et al., 1997; Ding et al., 1998, 2000, 2001; Vaziri and Ding, 2001; Vaziri et al., 1997, 1999a,b, 2000, 2001, 2003; Zhou et al., 2002; Ni et al., 2004) have published a number of articles relating to the production of ROS and alterations in enzymatic activities in Pb-induced hypertension. These were discussed in detail in Section 5.5 but are described briefly here. In the majority of studies, Pb-induced hypertension was produced by the administration of Pb acetate, 100 ppm in drinking water, for 3 months to male Sprague-Dawley rats. Early studies (Gonick et al., 1997) revealed that hypertension could occur in the absence of changes in NO or cGMP but with an attendant rise in plasma and kidney MDA-TBA, indicating an increase in ROS. In a second study, Ding et al. (1998) showed that infusion of arginine, the precursor of NO, or DMSA, a thiol Pb chelator and antioxidant, reduced blood pressure to or towards normal, while simultaneously increasing depressed urinary NO and decreasing an elevated MDA-TBA. Ding et al. (2000, 2001) further showed that the ROS species, $^{\bullet}OH$, measured as salicylate-trapped 2,3 dihydroxybutyric acid, was increased in plasma and cultured rat aortic endothelial cells after exposure to Pb, and that dimethylthiourea, a reputed

scavenger of $^{\bullet}OH$, returned blood pressure, MDA-TBA, $^{\bullet}OH$, and nitrotyrosine to or towards normal. Ni et al., in 2004, demonstrated in both human coronary endothelial (EC) and vascular smooth muscle cells (VSMC) that Pb acetate also increased superoxide (demonstrated by flow cytometry using hydroethidine) and H_2O_2 (demonstrated with dihydrorhodamine) production. After long-term (60-h) exposure, detectable superoxide levels fell to near normal while H_2O_2 production remained high.

Vaziri et al. (1997) showed that lazaroids, a class of non-thiol antioxidant, also restored blood pressure, NO, and MDA-TBA to normal. Vaziri et al. (1999a) studied rats treated for 12 weeks with either Pb acetate alone or Pb acetate + vitamin E-fortified food (5000 units/kg rat chow). They measured urinary excretions of stable NO metabolites (NO_x) and plasma and tissue abundance of nitrotyrosine, the footprint of NO oxidation by ROS. The Pb-treated group showed a marked rise in blood pressure; a significant increase in plasma and kidney, heart, liver, and brain nitrotyrosine abundance; and a substantial fall in urinary NO_x excretion. Concomitant administration of high-dose vitamin E ameliorated hypertension and normalized both urinary NO_x excretion and tissue nitrotyrosine without altering tissue Pb content. Vaziri et al. (1999b) also measured eNOS and iNOS in the aorta and kidney of Pb-treated and Pb + vitamin E-treated rats. Lead treatment increased both isotypes in aorta and kidney, signifying increased NO production, while Pb + vitamin E lowered aortic, but not kidney, expression of eNOS and iNOS. Vaziri and Ding (2001) tested the effect of Pb, 1 ppm, on cultured human EC cells. Lead was tested alone or with either the SOD-mimetic agent, tempol, or a potent antioxidant lazaroid compound (both at 10^{-8} or 10^{-7}mol/L) on eNOS expression and NO production. Lead-treated cells showed a significant upregulation of endothelial eNOS, increase in protein abundance, and increase in the production of NO metabolites. Treatment with either tempol or lazaroids abrogated the Pb-induced upregulation of eNOS protein and NO_x production. Vaziri et al. (2001) also studied increases in NOS isoforms in vivo in Pb-induced hypertension and reversal by tempol. Both eNOS and iNOS were increased in kidney, aorta, and heart, while NOS was increased in cerebral cortex and brain stem, of Pb-treated rats; blood pressure and NOS isoforms were returned to normal by tempol. Vaziri et al. (2003) determined whether the oxidative stress in animals with Pb-induced hypertension is associated with dysregulation of the main antioxidant enzymes (i.e., SOD, catalase, and GSHPx), or increases in the superoxide-producing enzyme NAD(P)H oxidase. At the conclusion of the experiment, immunodetectable CuZnSOD,

MnSOD, catalase, GSHPx, and the gp^{91}phox subunit of NAD(P)H oxidase were measured by Western analysis in the kidney, brain, and left ventricle of control and Pb-exposed rats. Lead exposure resulted in a significant increase in kidney and brain CuZnSOD with a significant increase in brain, and insignificant increase in kidney and heart, gp^{91}phox. In contrast, MnSOD, catalase, and GSHPx in the kidney, brain, and left ventricle were unchanged. Incubation with Pb acetate did not alter SOD activity in vitro. Thus, animals with Pb-induced hypertension exhibited oxidative stress, which was associated with mild upregulation of the superoxide-generating enzyme NAD(P)H oxidase, with no evidence of quantitative SOD, catalase, or GSHPx deficiencies.

Vaziri et al. (2000) demonstrated that induction of oxidative stress in normal animals (by feeding the GSH synthase inhibitor, buthionine sulfoximine, 30 mmol/L in drinking water for 2 weeks) led to an increase in blood pressure, a reduction of urinary NO_x, a 3-fold decrease in liver GSH, and an increase in nitrotyrosine in kidney, aorta, heart, liver and plasma. The administration of vitamin E + ascorbic acid ameliorated hypertension and also mitigated nitrotyrosine accumulation despite persistent GSH depletion. This experiment demonstrated the importance of GSH in protecting against the adverse effects of ROS accumulation in normal animals. The majority of the studies reported by Vaziri and co-workers indicated that low Pb exposure induced hypertension to be primarily mediated by ROS-induced depletion of NO. NO production, on the other hand, is stimulated, as shown by the increase in eNOS and iNOS. Enzymatic control of ROS levels by low Pb is achieved by upregulation of NAD(P)H oxidase with no decrease in SOD, catalase, or GSHPx, i.e., the enzymes that breakdown ROS. Scavengers of ROS ameliorate the elevated blood pressure, while the depletion of the endogenous methyl scavenger, GSH, increases blood pressure in normal animals. No studies to date have addressed the question of why high-dose Pb administration does not lead to hypertension.

Farmand et al. (2005) pursued enzymatic studies by activity measurements and measures of protein abundance in the rat kidney and aorta when rats are fed Pb acetate 100 ppm for 12 weeks. They demonstrated that the activities of CuZnSOD and catalase were increased by Pb administration in renal cortex and medulla, whereas GSHPx was unchanged. In the thoracic aorta, Pb exposure resulted in significant upregulation of CuZnSOD activity, while catalase and GSHPx activities were unchanged, CuZnSOD, MnSOD, and catalase protein abundance were

likewise unchanged. However, guanylate cyclase protein abundance in the thoracic aorta was decreased. The authors suggested that the Pb-induced compensatory upregulation of CuZnSOD and catalase and the decrease in aortic guanylate cyclase may be related to Pb-induced hypertension.

Gurer et al. (1999a) evaluated whether captopril, an ACE inhibitor, acted as an antioxidant in Pb-exposed F344 rats. Lead acetate was given in drinking water for 6 weeks. Group I were the controls; group II received 1100 ppm Pb for 5 weeks and plain water during the week 6; group III received 1100 ppm Pb for 5 weeks and, during the week 6, received water containing captopril (10 mg/day). Blood Pb concentrations in the control group measured 0.8 µg/dL; in the Pb treated group, 24.6 ± 20 µg/dL; and in the Pb + captopril group, 23.8 ± 1.6 µg/dL. MDA concentrations in liver, brain, and kidney were increased by Pb administration and reduced to or towards normal by the Pb + captopril treatment. GSH concentrations were decreased by Pb administration and restored by Pb + captopril treatment, whereas GSSG concentrations were increased by Pb administration and reduced by Pb + captopril treatment. Thus, this study showed that captopril was capable of augmenting the reducing capacity of the cells by increasing GSH/GSSG ratios without affecting blood Pb concentrations.

McGowan and Donaldson (1987) examined total nonprotein sulfhydryl and GSH concentrations in liver and kidney as well as GSH-related free amino acid concentrations in liver, kidney, and plasma in 3-week-old Pb-treated (2000 ppm dietary lead) chicks. Cysteine, converted from methionine, is the rate-limiting amino acid in GSH formation. The availability of glutamate, cysteine, and glycine becomes important in the restoration of depleted GSH. GSH, nonprotein sulfhydryl groups, glycine, and methionine were increased versus controls in the liver, but only nonprotein sulfhydryl, glycine, cysteine, and cystathionine increased in the kidney. Plasma levels of cysteine, taurine, and cystathione were reduced. Thus, Pb, for short periods of time, increases GSH turnover. These and other related studies are summarized in Table AX5-7.2.

Effect of Lead on Selective Renal Enzyme Levels

Effects of Lead on Renal NAG

Dehpour et al. (1999) studied NAG release by the rat kidney perfused with Pb acetate at 10, 20, and 50 µg/dL for 120 min, or Pb + arginine (the substrate for NO), or Pb + L-NAME

(an inhibitor of NOS). Lead acetate caused a time and concentration-dependent increase in enzymuria. Addition of arginine decreased, while addition of L-NAME increased, Pb-induced NAG release. Histologic studies showed damage to some of the proximal tubule epithelial cells in rats treated with 50 µg/dL Pb acetate, damage that which was increased further by the addition of L-NAME.

Effect of Lead on Renal GST

Two studies have evaluated the effects of Pb administration on GST isoforms in developing rat kidney. In the first study (Moser et al., 1995), rats were treated either acutely (14- and 50-day old rats given three daily injections of Pb acetate, 114mg/kg) or chronically (Pb levels of 0, 50, 250, and 500 ppm in drinking water for 1, 2, 3, 4, and 7 weeks postnatal). Chronic treatment rats were also given a 0.66% low calcium diet or standard rat chow. Essentially all kidney cytosolic GSTs (Yb1, Yb2, Yp, Yc1, Yl, Yb3, Ya1, Ya2, Yk) increased in the acute experiment (1.1- to 6.0-fold). In the chronic experiment, all but one isoform (Yb3) increased, and these results were markedly exacerbated by placing the rats on a low-calcium diet (Yb1 and Yp increased >25-fold). In the second study (Oberley et al., 1995), pregnant rats were given 250 ppm Pb from conception until weaning, then pups received 500 ppm from weaning until termination at either 3 or 7 weeks of age. By 7 weeks, proximal tubular cells showed intranuclear inclusions, tubular injury, and interstitial fibrosis. Creatinine clearances were reduced (0.55 + 0.05 versus 1.05 + 0.07 mL/min/100g; $P < 0.001$). Treatment with Pb also caused large increases in the immunoreactive protein of Yc, Yk, Yb1, and Yp GST subunits in proximal tubules but did not increase in the antioxidant enzymes CuZnSOD, catalase, and GSHPx.

Another experiment that examined the effect of an acute dose of Pb as Pb nitrate (100 µmol/kg IV) on GST levels in rat liver and kidney was reported by Planas-Bohne and Elizade (1992). Seventy hours after injection, there was a marked increase in GST activity in both organs, accompanied by induction of the isoenzyme GST 7-7 in the liver.

The relationship between GST induction by acute exposure to Pb acetate and oxidative stress was explored by Daggett et al. (1998). Rats in the 72-h and 7-day experimental groups received three consecutive daily injections of 114 mg/kg body weight of Pb acetate. The level of kidney GST was increased at 3, 6, 12, and 24 h after injection, but MDA levels remained

unchanged. Immunohistochemical markers of oxidative stress and NO production (MnSOD, eNOS, iNOS, and 4-hydroxy-2-nonenal) also did not change. The authors concluded that the GST changes were not the result of oxidative stress.

Witzman et al. (1998) and Kanitz et al. (1999) utilized two-dimensional (2-D) gel electrophoresis to explore protein markers of Pb exposure. Witzman et al. (1998) gave three consecutive IP injections of Pb acetate (114 mg/kg) to Sprague-Dawley rats, sacrificed them on the fourth day, and subjected the cytosolic fraction of kidney homogenate to 2-D gel electrophoresis. Lead exposure caused detectable inductions in both GSTP1 and GSTM1 and caused quantifiable charge modifications in GSTP1. Kanitz et al. (1999) examined kidney protein expression in male rabbits injected with Pb acetate (260, 360, or 100 µg/kg) designed to produce blood Pb levels of 20, 40, or 80 µg/dL. Injections were given during weeks 6 to 10, followed by maintenance doses during study weeks 11 to 20. Kidney homogenates were subjected to 2-D electrophoresis. Significant quantitative changes occurred in 12 proteins in a dose-related manner. Four proteins cross-reacted with anti-rat GSTp1 (π-GST). Thus, both studies confirmed GST induction by Pb.

Daggett et al. (1997) examined the effects of triethyl Pb administration on the expression on GST isoenzymes and quinone reductase in rat kidney and liver. Fischer 344 rats were given one IP injection of triethyl Pb chloride (10 mg/kg body weight) and subsequent changes in enzyme expression were measured. There was a significant increase in GST activity in kidney; and all GST subunits were significantly elevated, the largest increase being a 3.2-fold increase in GST Yb1. In the liver, injection of triethyl Pb chloride resulted in decreased GST activity. The largest decrease in subunits was a 40% reduction in GST Ya1. The activity of quinone reductase was elevated 1.5-fold in kidney and 2.7-fold in liver within 14 days after the injection of triethyl Pb chloride.

Effects of Lead on Renal Heme Enzymes

Vij et al. (1998) explored Pb-induced alterations in male rats in the heme synthesizing enzymes, ALAD and uroporphyrinogen I synthetase, and also the effect of ascorbic acid supplementation in reversing these alterations. Lead-treated rats were injected IP with 20 mg/kg of Pb acetate for 3 consecutive days and sacrificed 4 days later. A separate group of animals was administered 100 mg/kg ascorbic acid PO for 3 days following Pb administration. Blood Pb

concentration was 4.7 ± 1.5 µg/dL in control rats, 16.6 ± 4.7 µg/dL in Pb-treated rats, and 7.8 ± 2.0 µg/dL in the Pb + ascorbic acid treated rats. Lead content of liver and kidney followed the same pattern. Blood ALAD activity was diminished in the Pb-treated rats but was restored in the Pb + ascorbic acid-treated rats. Uroporphyrinogen I synthetase activity followed the same pattern in blood but was not restored by ascorbic acid in liver. Total and nonprotein sulfhydryl concentrations in blood were depressed by Pb administration and were not restored by ascorbic acid. However, levels in liver and kidney were restored by ascorbic acid.

ALAD levels following administration of Pb were also investigated by Rodrigues et al. (1996a) and Peixoto et al. (2004). The study by Rodrigues et al. (1996a) examined rats from Pb-exposed mothers that were maintained after weaning on either 0.5 or 4.0 mM Pb acetate in drinking water for 21 days or 6 months. At sacrifice, ALAD activity was measured in kidney, forebrain, and cerebellum. Both 6-month-old Pb-exposed groups showed an increase in the kidney-to-body weight ratio, suggesting Pb-induced cell proliferation in the kidney. Blood Pb increased from 6.5 to 7.6 µg/dL in the 21-day-old exposed rats compared to 6-month-old controls. In the 0.5 mM Pb-treated group, blood Pb was 9.8 µg/dL in the 21-day-old and 41.6 µg/dL in 6-month-old rats, while in the 4.0 mM group, blood Pb was 44.4 µg/dL in the 21-day-old and 116.9 µg/dL in the 6-month-old group. ALAD activity was reduced at 6 months in the forebrain of the 4.0 mM Pb-treated group, and in the kidneys at 6 months in both the 0.5 mM and 4.0 mM Pb-treated groups. The study by Peixoto et al. (2004) examined the in vitro sensitivity (IC_{50}) to Pb of ALAD activity of brain, kidneys, and liver from suckling rats aged between 1 and 5, 8 and 13, or 17 and 21 days. The metal concentrations ranged from 0 to 50 µM for Pb acetate. Rats in the first age group showed the greatest sensitivity in all three organs. Liver was the least sensitive to ALAD inhibition by Pb, while brain was the most sensitive.

Effects of Lead on NaK-ATPase

Fox et al. (1991b) explored the effect of in vivo Pb exposure on adult rat retinal and kidney NaK-ATPase. Pups, exposed to Pb through the milk of dams consuming 0, 0.02, or 0.2% Pb solutions, had mean blood Pb concentrations of 1.2, 18.8, and 59.4 µg/dL at weaning, respectively, and 5 to 7 µg/dL as 90 to 100-day-old adults. Prior Pb exposure produced significant dose-dependent decreases in isolated retinal NaK-ATPase activity (−11%; −26%), whereas activity in the kidney was unchanged. In contrast, NaK-ATPase from both isolated

control tissues was inhibited by Pb in vitro. The half-maximal inhibitory dose of Pb for retinal and renal NaK-ATPase was 5.2×10^{-7} and 1.3×10^{-5} M, respectively. Retinal and renal NaK-ATPase were 20-fold and 1.1-fold more sensitive to inhibition by Pb than calcium. The increased sensitivity of retinal, compared to renal, NaK-ATPase to inhibition following in vivo or in vitro Pb exposure may be related to their different α subunit composition.

Kramer et al. (1986) had also explored the half-maximal inhibitory dose for Pb chloride on renal cortical homogenate NaK-ATPase, and found it to be 7×10^{-5} M. There was a competitive inhibition with regard to the substrate, ATP. Of several metals tested, Pb was second only to Hg in potency as a NaK-ATPase inhibitor.

Weiler et al. (1990) studied the effect of Pb on the kinetics of purified (from hog cerebral cortex) NaK-ATPase and potassium-stimulated p-nitrophenylphosphatase (K-pNPPase), which is referred to as the E2 configuration of the NaK-ATPase system. IC_{50} for Pb was found to be 8.0×10^{-5} M for NaK-ATPase and 5.0×10^{-6} M for K-pNPPase. Inhibition of NaK-ATPase by Pb was found to be noncompetitive with respect to K, but competitive with respect to Na and MgATP. Inhibition of K-pNPPase by Pb was competitive with respect to K.

Effects of Lead on Cardiovascular Hormones

Effects of Lead on Endothelin

Khalil-Manesh et al. (1993a) examined the role of endothelial factors in Pb-induced hypertension. They found that low Pb administration (0.01%), but not high Pb administration, (0.5%) resulted in increased blood pressure in rats treated for 12 months. In the low-Pb-treated rats, measurement of plasma endothelins-1 and -3 revealed that endothelin-3 concentration increased significantly after both 3 months (Pb, 92.1 ± 9.7 versus control, 46.7 ± 12.0 pmol/mL; $p < 0.001$) and 12 months (Pb, 105.0 ± 9.3 versus control, 94.1 ± 5.0 pmol/mL; $p < 0.01$), while endothelin-1 was unaffected. Plasma and urinary cyclic GMP concentrations, as a reflection of endothelium-derived relaxing factor (EDRF), decreased significantly at 3 months (plasma Pb, 1.8 ± 0.9 versus control, 4.2 ± 1.6 pmol/mL; $p < 0.001$) and 12 months (plasma Pb 2.2 ± 0.7 versus control, 4.2 ± 0.9 pmol/mL; $p < 0.001$). High levels of Pb exposure did not result in hypertension, perhaps related to the fact that plasma concentrations of endothelin-1, endothelin-3, and cyclic GMP were unaltered at 3 months, while their concentrations were significantly decreased at 12 months (plasma cyclic GMP at 12 months, 2.2 ± 0.7, Pb, versus

4.2 ± 0.9 pmol/mL, control; $p < 0.001$). Thus, the path to development of hypertension in low-Pb rats was thought to be through an increase in the concentration of the vasoconstrictor, endothelin-3, and a decrease in the vasodilator hormone, endothelium-derived relaxing factor or NO.

Novak and Banks (1995) studied the effects of Pb on the actions of endothelin. They measured renal clearances and mean arterial pressure in rats in which endothelin-1 was infused at 110 ng/kg/min for 30 min. Lead was infused as Pb acetate throughout the experiment at 0.48, 4.8, and 24 nmoles/min. At the two higher doses, Pb significantly attenuated the endothelin-induced increase in mean arterial pressure; Pb infused as 0.48 nmoles/min had no effect. An endothelin-induced decrease in GFR in control rats was completely blocked at the higher doses of Pb. In additional experiments, calcium chloride was infused at 500 nmoles/min for 105 min, and then calcium + Pb (4.8 nmoles/min) were infused for another 105 min. In these experiments, there was no Pb-induced inhibition of the mean arterial pressure response to endothelin. However, the GFR response to the peptide remained blocked. These data illustrate that Pb inhibits the cardiorenal actions of endothelin and that a calcium-related process is involved in the systemic, but not the renal, component of this inhibition.

Effects of Lead on the Catecholamine System

Carmignani et al. (2000) studied the effects of 10 months of low Pb exposure (60 ppm of Pb acetate), on catecholamine and monoaminoxidase (MAO) levels. Plasma catecholamines were measured by HPLC and MAO in aorta, liver, heart, kidney, and brain by a histochemical technique. Plasma norepinephrine (NE) increased by 104% and adrenaline by 81%, with no changes noted in L-DOPA and dopamine levels. MAO activity was increased in all organs. These workers ascribed the low Pb-induced hypertension in part to raised catecholamines levels.

Tsao et al. (2000) and Chang et al. (2005) measured changes in the β-adrenergic system in Wistar rats during and following Pb exposure. In Tsao et al. (2000), rats were chronically fed with 0.01, 0.05, 0.1, 0.5, 1.0, and 2.0% Pb acetate for 2 months. Plasma catecholamine levels were measured by HPLC; cAMP levels in heart, kidney, and aorta by radioimmunoassay; and β-adrenergic receptors in heart, kidney, and aorta membranes by a radio ligand binding assay. Blood Pb increased from 0.05 ± 0.05 μg/dL in controls to 85.8 ± 4.1 μg/dL in the 2.0% Pb-treated group. Plasma NE, but not E, levels increased with increasing Pb dosage.

β-adrenoreceptor density of heart and kidney decreased progressively with increasing Pb dosage, whereas kidney β-adrenoreceptor density increased up to the 0.5% Pb group and then remained constant. Unstimulated cAMP was constant in all tissues, but cAMP stimulated by isoprotorenol was lowered progressively in aorta and heart and increased in kidney. Chang et al. (2005) continued these measurements in rats fed 2% Pb acetate for 2 months then withdrawn from Pb for periods of 1, 2, 3, 4, 5, 6, and 7 months. Blood Pb levels, systolic and diastolic blood pressure levels, and plasma NE were reduced after cessation of Pb exposure. This occurred in conjunction with an increase in β-adrenoreceptor density in heart and aorta and a decrease in β-adrenoreceptor density in kidney. (See Table AX5-5.5 for details on these studies).

Effects of Chelators (Single or Combined) on Lead Mobilization

These studies are summarized in Tables AX5-7.3 and AX5-7.4. For the sake of brevity, they will not be discussed further here.

Effects of Other Metals on Lead Distribution

Lead and Calcium

Fullmer (1992) published a review of intestinal interactions of Pb and calcium. High affinity Pb binding to intracellular calcium receptors and transport proteins, as well as the involvement of Pb in calcium-activated and calcium-regulating processes, are thought to provide a partial molecular basis for the cellular and systemic effects of Pb.

Maldonado-Vega et al. (1996) examined the intestinal absorption of Pb and bone mobilization during lactation. All experiments were started with 3-week-old female Wistar rats. Rats were impregnated at 16 weeks and were fed a 100 ppm solution of Pb acetate for 158 or 144 days (mid-lactation or before lactation). Rats were also exposed for only 14 days, from 144 to 158 days (i.e., only during lactation). Nonpregnant rats from the same litter were exposed to Pb for periods equivalent to each of these groups. In the nonpregnant rats, blood Pb increased to 27.3 μg/dL from 5.2 μg/dL in controls. Similarly, kidney Pb increased to 13.2 nmol/g from 0.5 nmol/g, and bone Pb increased to 88.9 nmol/g from 0.9 nmol/g. ALAD activity decreased to 410 nmol/h/mL from 1004 nmol/h/mL. Compared to nonpregnant rats, there was a moderate increase in blood Pb in the lactating animals whether the Pb was given to mid-lactation or up to the period before lactation. Similarly, when Pb was administered only during lactation, there

was a much higher increase in blood Pb in the pregnant rats than in the nonpregnant rats. Bone Pb concentration increased when Pb was given only during lactation, whereas bone Pb decreased (compared to Pb-treated nonpregnant rats) when the Pb was given either before lactation or before and during lactation. The authors considered that resorption of Pb from bone was the main additional source of Pb during lactation. The data indicate that Pb stored in bone as a result of prior maternal exposure should be considered as a major source of self intoxication and of Pb in milk available to suckling pups.

Lead and Cadmium

Skoczyńska et al. (1994) compared the effects of the combined exposure to Pb and cadmium to each metal singly on tissue composition of trace metals. Experiments were performed on 5- to 6-week-old male Buffalo rats given Pb acetate (70 mg Pb/kg body weight twice a week) and cadmium chlorate (20 mg Cd/kg body weight once a week) intragastrically for 7 weeks either singly or in combination. Blood Pb in the control group was 5.1 µg/dL, compared to 29.6 µg/dL in the Pb-treated group. In contrast, the Pb + cadmium group showed a blood Pb of 37.4 µg/dL. After combined exposure to Pb and cadmium, the level of these metals in the liver and kidney was lower than after the single administration of Pb or cadmium. Exposure of the rats to cadmium resulted in an increase of kidney zinc and copper and liver zinc levels; combined exposure to Pb + cadmium did not produce more extensive changes in tissue zinc and copper concentrations.

Lead and Selenium

Othman and El Missiry (1998) examined the effect of selenium against Pb toxicity in male rats. Male albino rats were given a single dose of Pb acetate (100 µmol/kg body weight) and sacrificed 3 or 24 h later. Another group of animals was pretreated with sodium selenite (10 µmol/kg body weight) 2 h before receiving Pb acetate and sacrificed 24 h later. Selenium is well known as an antioxidant and cofactor for GSHPx. In this experiment, GSH content, GSHPx, SOD activities, and the products of lipid peroxidation (i.e., TBARS) were determined. It was found that lipid peroxidation was prevented and the reduction in GSH caused by Pb in liver and kidney was diminished by selenium. Lead-induced diminution in SOD activity and GSHPx activity was also returned to normal by selenium.

Tandon et al. (1992) studied the effect of selenium supplementation during chelation of Pb with $CaNa_2EDTA$. Rats were given Pb acetate 10 mg/kg/day by gastric gavage for 6 weeks. This was followed by a 5-day treatment course of $CaNa_2EDTA$, 0.3 mmol/kg IP or of $CaNa_2EDTA$ + sodium selenite, 0.5 mg/kg PO. Selenium had marginal effects on Pb removal by $CaNa_2EDTA$ in blood, liver, and kidney and similar effects on ALAD activity.

Lead and Zinc

Flora et al. (1989) examined the role of thiamine, zinc, or their combination in the prevention or therapy of Pb intoxication. Albino rats received the following treatments daily through gastric gavage for 6 days each week over a six-week period: 10 mg/kg of Pb as Pb acetate; or the same dose of Pb acetate + thiamine (25 mg/kg) zinc sulfate (25 mg/kg) or Pb + thiamine and zinc. Rats exposed to Pb only were additionally divided into four groups treated by gastric gavage daily for 6 days as follows: group I, water only; group II, thiamine only; group III, zinc only; and group IV, combined zinc + thiamine. The activities of blood ALAD, blood ZPP, blood Pb, and urine ALA were determined. Blood Pb concentrations increased from 6.2 to 120.9 µg/dL, contrasting normal controls with Pb-treated animals. There was a slight reduction in blood Pb in animals treated with either thiamine or zinc and a greater reduction in animals treated with thiamine + zinc. In the post-Pb-exposure treatment group, thiamine + zinc was also the most effective treatment. Liver and kidney Pb levels followed the same course, but brain Pb was not reduced by treatment. Blood ALAD activity was decreased from a normal level of 7.63 µmol ALA/min/L to 0.69 in Pb-treated animals and restored to 7.52 in Pb + thiamine + zinc-treated rats. ZPP was increased from 1.78 µg/g hemoglobin to 4.22 in Pb-treated animals and reduced to 2.50 in Pb + thiamine + zinc-treated animals. Urine ALA increased from 0.07 to 0.24 mg/dL in Pb-treated animals but decreased to 0.17 in the Pb + thiamine + zinc-treated rats. Prevention was more effective than post-Pb-exposure treatment. This was thought to be due mainly to the decrease in the absorption of Pb in the GI tract in the presence of thiamine and/or zinc.

Flora et al. (1994) explored the dose-dependent effects of zinc supplementation during chelation of Pb in rats. The chelator employed was $CaNa_2EDTA$, whose toxic effects are known to be mainly due to the depletion of endogenous zinc and, possibly, copper and manganese. Male Wistar rats were started on exposure to Pb acetate, 10 mg/kg, administered through gastric

gavage once daily for 56 days. Twenty-four hours later, the Pb-exposed animals were treated daily for 5 days as indicated: group I, saline ; group II, $CaNa_2EDTA$ 0.3 mmol/kg, IP, once daily for 5 days; group III, $CaNa_2EDTA$ + zinc sulfate, 10 mg/kg, PO once daily for 5 days; and group IV, $CaNa_2EDTA$ + zinc sulfate, 50 mg/kg, PO once daily for 5 days. Blood ALAD decreased from 6.30 to 1.44 nmol/min/mL erythrocyte in Pb-exposed animals, with no change after $CaNa_2EDTA$ treatment and partial restoration after the $CaNa_2EDTA$ + zinc, 10 mg/kg treatment. There was no improvement following zinc, 50 mg/kg. Blood Pb concentration increased from 4.6 µg/dL to 43.0 µg/dL in Pb exposed animals, decreased to 22.5 µg/dL in $CaNa_2EDTA$-treated animals and decreased further to 16.5 µg/dL in $CaNa_2EDTA$ plus zinc-treated animals. Zinc at 50 mg/kg led to an increase in blood Pb to 56.1 µg/dL. Changes in the liver followed the same pattern, whereas zinc increased the Pb levels further in the kidney, and zinc had no influence on Pb content in the femur. Blood zinc decreased from 6.1 to 5.7 µg/mL in Pb-exposed rats and further to 5.0 µg/mL in $CaNa_2EDTA$-treated animals. There was an increase to levels of 6.6 µg/mL on the 10 mg/kg supplement of zinc and a further increase to 8.1 µg/mL on the 50 mg/kg zinc supplement.

Lead and Iron

Hashmi et al. (1989) examined the influence of dietary iron deficiency, Pb exposure, or the combination of the two on the accumulation of Pb in vital organs of rats. Animals fed an iron deficient diet for 2 weeks were also subjected to orbital plexus puncturing twice a week to allow a Hb levels to decrease to 7 to 8 g/dL. Animals were thereafter treated for the next 6 weeks with iron deficient diets or iron-deficient diets + 0.1% Pb acetate in drinking water. At the end of 3 and 6 weeks, animals from each group were sacrificed. Feeding of an iron-deficient diet during Pb exposure enhanced the accumulation of Pb in soft tissues and flat bones. For example, liver Pb content was 0.75 µg/g in control animals, 8.43 in Pb treated animals, and 12.93 in iron-deficient and Pb-treated animals. The sequence of events was similar in kidney, spleen, and femur except that the Pb content in femur was reduced in the iron deficient and Pb-treated group.

Singh et al. (1991) conducted a study to ascertain the role of iron deficiency during pregnancy in inducing fetal nephrotoxicity in mothers exposed to Pb. Rats were fed either a normal iron diet or an iron free synthetic diet for 15 days, followed by a diet containing half of the daily required iron (47 mg/100 g ferrous ammonium sulfate) for a further 15 days. Female

animals were mated with healthy adult males. Lead doses of 250, 500, 1000, and 2000 ppm were given in drinking water during pregnancy and lactation. Fetuses were removed by Caesarean section on the 21st day. Maternal blood Pb levels in rats on an iron deficient diet were higher than those in rats on a normal iron diet at all Pb dosing levels. Similarly, placental Pb levels were higher in animals on an iron-deficient diet as compared to a normal diet. Lead content in the fetuses were higher on the iron-deficient diet. Lead administration resulted in dose-dependent hydropic degeneration of renal proximal tubular cells in the fetuses. At a dose of 2000 ppm Pb with iron deficiency, more Pb accumulated in maternal blood, placenta, and fetuses and maximum pathological changes were seen in the fetal kidney as compared to other doses.

Lead and Aluminum

Shakoor et al. (2000) reported beneficial effects of aluminum on the progression of Pb-induced nephropathy in rats. Male albino rats were treated with water only or Pb acetate (125 mg/kg) and/or aluminum chloride (50 mg/kg or 100 mg/kg) for a period of 90 days. Aluminum was found to prevent the Pb-induced increase in relative kidney weight in a dose-dependent manner. Aluminum also prevented Pb-induced increases in plasma creatinine levels of Pb-treated animals. The net deposition of Pb in kidneys was lower in animals that were given both Pb acetate and aluminum chloride simultaneously. By day 90, plasma creatinine was 1.26 mg/dL in control animals, 1.88 mg/dL in Pb-treated animals, and 1.34 and 1.44 mg/dL in Pb- and aluminum-treated animals. Similarly, kidney Pb increased from 5.4 μg/g in control animals to 220.0 μg/g in Pb-treated animals and decreased to 138.5 and 98.9 μg/g in Pb- and aluminum-treated animals. These and other related studies are summarized in Table AX5-7.5.

Lead, Cadmium, and Arsenic

In their review of mechanisms of nephrotoxicity from metal combinations, Madden and Fowler (2000) discuss the effects of Pb, cadmium, and arsenic combinations, given that such combinations may be found in the industrial setting or at toxic dump sites. Cadmium has been shown to interact with Pb, minimizing Pb kidney effects by lowering the renal Pb burden and preventing the appearance of Pb inclusion bodies (Mahaffey et al., 1981). Thus, cadmium may therefore affect the binding of Pb to Pb-binding protein (Mistry et al., 1985). Lead, cadmium,

and arsenic combinations also increase the degree of porphyrinuria beyond that produced by Pb alone (Fowler and Mahaffey, 1978).

5.7.4.4 Effect of Age on Lead Toxicity

Han et al. (1997) examined the hypothesis that the high rate of bone remodeling during childhood and the consequent high calcium and Pb turnover would result in a substantial reduction in bone Pb stores, so that much of the Pb incorporated in bone during childhood does not persist into adulthood. They treated female Sprague-Dawley rats with 250 ppm of Pb in drinking water for 5 weeks beginning at 5, 10, or 15 weeks of age. Organ harvesting occurred 4 weeks after the end of Pb exposure for all groups, as well as 8 and 20 weeks after cessation of Pb ingestion in the rats exposed beginning at 5 weeks of age. Organs examined were brain, kidney, liver, femur, and spinal column bone. Blood and organ Pb concentrations were significantly higher in the rats exposed beginning at 5 weeks of age than in those exposed beginning at 10 or 15 weeks of age. The results of this experiment rejected the hypothesis and suggested instead that a younger age at Pb exposure is associated with greater Pb retention and toxicity, even in the absence of continued Pb exposure.

Garcia and Corredor (2004) examined biochemical changes in the kidneys after perinatal intoxication with Pb and/or cadmium. Lead acetate (300 ppm) and/or cadmium acetate (10 ppm) were administered in drinking water to pregnant Wistar rats from day 1 of pregnancy to parturition (day 0) or until weaning (day 21). The following kidney enzyme activities were determined: alkaline and acid phosphatases, Mg-ATPase, and NaK-ATPase. Blood Pb was measured in control pups as well as in pups exposed to Pb at parturition and at weaning. Control pups showed 1.43 µg/dL of blood Pb compared to 31.5 µg/dL at day 0 and 22.8 µg/dL at day 21 in pups exposed to Pb. In those rats receiving both cadmium and Pb, the blood Pb concentration was 23.2 µg/dL at day 0 and 13.2 µg/dL at day 21. Lead caused a significant inhibition of kidney alkaline phosphatase and kidney acid phosphatase. At parturition, Pb intoxication produced a strong inhibition of NaK-ATPase (~80%) as well as of Mg-ATPase activities (~24%); whereas, when Pb was given in combination with cadmium, these inhibitory effects were attenuated. At weaning, Pb continued to produce a significant inhibition of Mg-ATPase but had no effect on NaK-ATPase. Thus, simultaneous perinatal administration of both Pb and cadmium seemed to protect against the toxicity produced by Pb separately.

Cory-Slechta (1990b,c) published two articles on the effects of old age on the disposition of Pb. In the study (1990c) male F344 rats, at the ages of 8 months (adult) and 16 months (old) were exposed to concentrations of 0, 250, or 500 ppm Pb acetate in drinking water for 7 months. At these Pb doses, prior studies had indicated that blood Pb levels ranged from 60 to 90 µg/dL. Blood Pb, ZPP, and urinary ALA levels were determined after both 3 and 7 months of exposure. Organ weights, tissue Pb concentrations, and urinary excretion of Pb, calcium, copper, and zinc were examined after 7 months of exposure. Tissue Pb distribution was markedly altered in old rats: in bone and kidney, Pb levels were reduced while liver Pb was substantially increased. Blood Pb levels in adult and old rats were comparable at both measurement intervals, as was urinary Pb excretion at 7 months. Lead-induced elevation of ZPP exhibited differential changes between 3 and 7 months; values in adults declined, while levels in old rats increased or remained unchanged. In the adult group, Pb exposure increased calcium excretion primarily at the 500 ppm exposure level. In contrast, Pb exposure decreased urinary calcium excretion in old animals at the higher exposure level. No effects of either age or Pb exposure were detected in the comparison of adult versus old urinary excretion of zinc or copper.

In the second study, Cory-Slechta (1990b), young (21 days old), adult (8 months old), and (16 months old) rats exposed to 0, 2, or 10 mg of Pb acetate/kg per day for 9.5 months were evaluated. Differences in the tissue distribution of Pb with age included lower bone levels, but increased concentrations in brain, liver, and kidney. Differences in blood Pb levels over the course of exposure were not remarkable. Thus, these effects did not appear to reflect an enhanced Pb absorption from the GI tract with age. Instead, the bone changes may reflect enhanced bone resorption with a concurrent decline in bone apposition with age, combined with altered patterns of urinary Pb excretion over time, i.e., elevated urinary Pb at 3 and 6 months, but comparable Pb excretion at 9.5 months, as compared to young and adult rats.

5.7.5 Summary

Highlights of the previous 1986 Lead AQCD and of studies done between 1986 and the present are outlined in this section.

1986 Document

- In animal studies, nuclear inclusion bodies were found in proximal tubules, identified as 27 kDa or 32 kDa proteins in combination with Pb. Subsequently, a 63 kDa Pb-binding cytosolic protein was described in kidney.

- Swollen mitochondria, with diminished mitochondrial function, were found in the proximal tubules.

- Renal ALAD was the same in Pb-treated animals as in controls when GSH was present, but was reduced when GSH was absent.

Newer studies

- Experimental studies have shown that early effects of Pb on tubular cells are generally reversible, but with continued exposure, a chronic irreversible nephropathy is likely to ensue.

- Hyperfiltration, when compared to age- and sex-matched normal controls, was found in adults who had suffered from childhood Pb poisoning, in young occupationally exposed Pb workers in Korea, and in both low-Pb-treated rats and high-Pb-treated rats up to 3 months of exposure. This is paralleled in animal experiments by an increase in kidney weight.

- Various new urinary markers for Pb toxicity have been described. These include NAG, β2-microglobulin, α1-microglobulin, retinol binding protein, GST, lysozyme, γ-glutamyl transferase, alanine aminopeptidase, prostanoids, and brush border antigens. Information on these markers is voluminous, but, on review, only GST and α1-microglobulin seemed to be appropriate urinary markers. NAG, which has been most extensively investigated, appears in detailed-animal studies to be overly sensitive, increasing in low-Pb-treated animals, despite an absence of pathological changes on ultrastructural study. Both β2-Microglobulin, and possibly retinol binding protein, which are low-molecular weight proteins reabsorbed by the proximal tubule, appeared to be elevated only with high blood Pb levels (>80 μg/dL).

- Animal studies have implicated free radicals in the pathogenesis of Pb-induced hypertension and renal disease. A sequence of free radicals can be demonstrated in Pb-induced disease, as evidenced by an increase in superoxide radicals, hydroxyl radicals, hydrogen peroxide, and peroxynitrite, together with a diminution in GSH in liver, brain, and aorta. Nitric oxide is most commonly decreased (by free radicals) as is urinary cyclic GMP. Aortic guanylate cyclase is decreased. The enzyme responsible for an increase in the production of free radicals, NAD(P)H oxidase, is increased by Pb, whereas eNOS and iNOS, the enzymes involved in the production of nitric oxide, are also increased, attesting to the importance of free radical destruction of nitric oxide. Antioxidants reverse these changes and diminish blood pressure.

- Norepinephrine and epinephrine are increased by Pb administration, whereas β-adrenoreceptor density of heart and kidney are decreased. In a second study, norepinephrine, but not epinephrine, was increased by Pb.

- Various antioxidants have been used in conjunction with chelators, to both remove Pb from tissue and to diminish free radicals. Taurine, lipoic acid, arginine, ascorbic acid, vitamin E, thiamine, tempol, and lazaroids have been used in conjunction with DMSA, all improving free radical diminution.

- Metal combinations have also been employed to reduce tissue Pb and/or affect free radicals. Cadmium increases Pb in blood when both are given, but diminishes Pb in liver and kidney. Selenium, an antioxidant, improves both parameters, as does thiamine or L-lysine plus zinc. Iron deficiency increases intestinal absorption of Pb and the Pb content of soft tissues and bone. Aluminum decreases kidney Pb content and serum creatinine in Pb-intoxicated animals.

- Age also has an effect on Pb retention. There is higher Pb retention at a very young age and lower bone and kidney Pb at old age, attributed in part to increased bone resorption and decreased bone accretion.

5.8 EFFECTS ON BONE AND TEETH

5.8.1 Biology of Bone and Bone Cells

By weight, bone is composed of 28% collagen fibers (predominantly type I collagen) and 5% noncollagenous proteins (osteocalcin, osteonectin, and other proteoglycans), with crystals of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] making up the remaining 67%. In addition to providing mechanical support for the body and protection of vital organs, the skeletal system also functions in a metabolic capacity. Historically, bones have been classified as either long or flat based on their appearance, with long bones including limb bones, e.g., the femur and humerus, and flat bones including the bones of the skull, sternum, pelvis, and scapula. Long and flat bones originate by distinct methods of formation, endochondral and intramembranous, respectively, with long bones eventually using both processes. In endochondral bone formation, a mineralized, cartilaginous matrix precedes the transition into true bone, while in intramembranous formation, the bone forming cells create bone directly without the cartilaginous template.

Bone cells responsible for producing the bone matrix of collagen and ground substance are called osteoblasts. Several signaling factors including growth factors and hormones

influence pre-osteoblastic cells to differentiate into mature osteoblasts and subsequently synthesize and mineralize the extracellular matrix to form mature bone. It is during the process of bone mineralization that the Pb ion (Pb^{2+}) can become incorporated by substituting for the calcium ion (Ca^{2+}). The bone cells responsible for bone resorption are the osteoclasts. Osteoclasts, which are large and multicellular (4 to 20 cells), dissolve bone matrix and hydroxyapatite by synthesizing and releasing lysosomal enzymes and acidifying the extracellular surroundings. It is during the process of dissolving bone, or demineralization that Pb stored in bone can be released locally and into the general system.

Bone cell function may be compromised both directly and indirectly by exposure to Pb. Regulation of bone cells occurs by numerous local and systemic factors, including growth hormone (GH), epidermal growth factor (EGF), transforming growth factor-beta 1(TGF-β1), and parathyroid hormone-related protein (PTHrP). As discussed further below in this section, the presence of Pb can potentially interfere with each of these factors. The bones of the skeleton serve as the primary reservoir for calcium and phosphate in the body and help to maintain homeostasis of these ions in the serum through bone turnover or remodeling. Vitamin D [1,25-$(OH)_2D_3$] maintains the normal range of calcium in the serum by increasing the efficiency of calcium absorption in the intestines and facilitating differentiation of stem cells into osteoclasts, which break down bone and mobilize calcium (and Pb) stores. Parathyroid hormone (PTH), in turn, regulates the production of vitamin D in the kidney. Lead has been shown to interfere with the action of both of these hormones. Other substances influenced by Pb and discussed in this section are alkaline phosphatase, an enzyme necessary for mineralization of bones and teeth, and osteocalcin, a noncollagenous protein whose spatial and temporal pattern of expression suggests a role in bone mineralization. Both substances are also markers for osteoblast activity and, by default, bone formation. Alkaline phosphatase is a potential carrier of ionic calcium and is capable of hydrolyzing inhibitors of mineral deposition such as pyrophosphates.

5.8.2 Summary of Information Presented in the 1986 Lead AQCD

Lead has been shown to become localized and accumulate in bones and teeth, with accumulation beginning as early as fetal development. Lead administered to rats as a single dose results in blood Pb concentrations that are initially elevated, but rapidly fall as Pb is transferred

to bone or excreted. The dose of Pb administered does not apparently affect distribution to the various body compartments; however, the rate-limiting step in the clearance of Pb from rats and mice involves absorption into/clearance from the skeletal system. The loss of Pb from various organs and tissues follows first-order kinetics, except from bone. More absorbed Pb is retained by young animals compared with adult animals, leading to higher tissue levels. Moreover, once Pb is incorporated into the young animal's body, the long-term rate of retention is greater than that of adults. In Pb-exposed animals, Pb is distributed subcellularly, preferentially to the nucleus and mitochondrial fractions.

During lactation in mice, a redistribution of tissue Pb occurs (mobilization), resulting in the transfer of Pb and calcium from mother to pups via the milk and subsequent overall loss of Pb in the mothers. Lead transfer to suckling rats via mother's milk has been reported to be ~3% of the maternal body burden or more, if Pb exposure continues during lactation. Eight days after a single injection of Pb, the content of Pb in rabbit's milk was 8-fold higher than the maternal blood level, suggesting Pb transfer can occur against a concentration gradient. Transplacental transfer of Pb from mother to fetus also occurs in various animals.

In rats, a significant reduction of calcium in the diet leads to enhanced uptake of Pb into the bones and other tissues. In general, an enhanced uptake of Pb into tissues is also seen in rats fed diets deficient in iron, zinc, copper, or phosphorus, and in the presence of low or excess vitamin D.

5.8.3 Bone Growth in Lead-Exposed Animals

Lead is readily taken up and stored in the bone of experimental animals, where it can potentially manifest toxic effects that result in stunted skeletal growth. In experiments reported since the 1986 Lead AQCD, Hać and Krechniak (1996) determined uptake and retention of Pb in bone from rats exposed to plain water or water containing Pb acetate (41.7 to 166.6 mg/L) for 12 to 16 weeks. After 4 weeks, the skeletal Pb in animals receiving the lowest dose was almost 5 times higher than control animals (5.9 versus 1.2 µg Pb/g bone, respectively). Lead levels in bones from animals receiving 83.3 mg/L and 166.6 mg/L were dose-dependently higher at 11.7 and 17.0 µg Pb/g bone, respectively, after 4 weeks of exposure. All bone Pb levels were maintained essentially in a steady state until the completion of exposure, when all animals were placed on control water. Approximately 64% of Pb remained in the bones of rats in the

83.3 mg/L exposed group at 64 days postexposure. No blood levels of Pb were reported. Similarly, airborne Pb can be inhaled and subsequently incorporated into bone. Grobler and co-workers (1991) exposed 6-week-old rats to either "clean air" (0.05 μg Pb/m^3) or air containing 77 μg Pb/m^3 and found significant differences in the amount of Pb incorporated into the alveolar bones of the animals. After 70 days, a mean of only 0.2 μg Pb/g of bone dry mass was found in bone from control animals, while 16.9 μg Pb/g was present in bone from the 77 μg Pb/m^3 exposure group. Exposure to air containing 249 μg Pb/m^3 for 28 days or 1,546 μg Pb/m^3 for 50 days, resulted in mean values of 15.9 and 158 μg Pb/g dry weight of Pb incorporation into the bone, respectively, highlighting the fact that dose and length of exposure are determinates of amount of Pb contained in the bones of these animals. Blood Pb levels were 2.6 μg/dL in control animals and ranged from 11.5 μg/dL to 61.2 μg/dL in the experimental groups. The uptake of Pb by bone has the potential for immediate toxic effects on the cellular processes occurring during bone growth, development, and maintenance, with the additional potential for delayed toxicity from release of stored Pb during periods of normal or accelerated bone remodeling.

Numerous studies have examined growth suppression associated with developmental Pb exposure. Hamilton and O'Flaherty (1994) examined the effects of Pb on growth in female rats, and subsequently, on growth and skeletal development in their offspring. Administration of drinking water containing either 250 or 1,000 ppm Pb to weaning female rats for 49 days produced no alteration in growth rate in these future dams. Blood Pb levels prior to mating were 2.7 ± 0.6 μg/dL (control), 39.9 ± 3.5 μg/dL (250 ppm group), and 73.5 ± 9.3 μg/dL (1000 ppm group). The rats were then bred, with Pb exposure continuing through parturition and lactation. Lead did not affect gestation time nor Day 1 suckling body weight, however, pup body weight and tail length were subsequently decreased in both exposure groups. A 10% increase in tibial growth plate width and disruption of chondrocyte organization were observed in offspring from the high exposure group.

In male rats exposed to 100 ppm Pb in drinking water and a low calcium diet for up to one year, bone density was significantly decreased after 12 months, while rats exposed to 5,000 ppm Pb had significantly decreased bone density after 3 months (Gruber et al., 1997). Pb content of femurs was significantly elevated over the content of control rats at all time points (1, 3, 6, 9, and 12 months). Blood Pb levels ranged from 1 to 4 μg/dL in control animals, 17 to 29 μg/dL in low dose animals, and 45 to 126 μg/dL in high dose animals. Trabecular bone from the low dose

animals was significantly decreased from 3 months forward. Young female rats exposed to 17 mg of Pb acetate per kg of feed for 50 days showed no differences in the length of the femurs, but the mean length of the 5th lumbar vertebra was significantly decreased (González-Riola et al., 1997; Escribano et al., 1997). The mean length of the femur growth plate cartilage was also significantly decreased in Pb-exposed animals. Blood Pb levels were not reported.

In a dose-response study, Ronis et al. (1998a,b) exposed pregnant rats to Pb acetate in drinking water (0.05% up to 0.45% w/v) beginning at gestation Day 5 and continuing through weaning of offspring at Day 21. Early bone growth was significantly depressed in a dose-dependent fashion in pups of all Pb-exposed groups, with growth suppression in male offspring considerably greater than in females. Significant decreases in plasma insulin-like growth factor and plasma sex steroids and increased pituitary growth hormone were also observed. Blood Pb levels in offspring ranged from 49 ± 6 µg/dL (0.05% group) to 263 ± 28 µg/dL (0.45% group). This is somewhat in contrast to the findings of Camoratto and co-workers (1993), who reported low exposure to 0.02% Pb nitrate (125 ppm Pb) did not significantly affect growth, though males weighed significantly less than females. Note however that the blood Pb levels in the rat pups were less (43.3 ± 2.7 µg/dL at 5d and 18.9 ± 0.7 µg/dL at 49d) than in the Camorrato study. Between age 57 and 85 days, Ronis et al. (1998b) noted that growth rates were similar in control and Pb-exposed pups, suggesting exposure at critical growth periods such as puberty and gender may account for differences in growth reported by various investigators. In a series of follow-up experiments, Ronis et al. (2001) reported a dose-dependent decrease in load to failure in tibia from Pb-exposed (0.15% and 0.45% Pb acetate in drinking water) male pups only. Hormone treatments (estradiol in females or L-dopa, testosterone or dihydrotestosterone in males) failed to attenuate Pb deficits during the pubertal period. Distraction osteogenesis experiments performed after stabilization of endocrine parameters (at 100 days of age) found decreased new endosteal bone formation and gap x-ray density in the distraction gaps of Pb-exposed animals (Ronis et al., 2001). Again blood Pb levels were high, ranging from 67 to 388 µg/dL in the offspring.

Hamilton and O'Flaherty (1995) found Pb disrupted mineralization during growth when they implanted demineralized bone matrix subcutaneously into male rats. In the matrix that contained 200 µg Pb/g of plaque tissue, alkaline phosphatase activity and cartilage mineralization were absent, though calcium deposition was enhanced. Separate experiments

found enhanced calcification and decreased alkaline phosphatase activity in rats implanted with a control (no Pb) matrix and given 1,000 ppm Pb in drinking water for 26 days (blood Pb 96.4 to 129.8 µg/dL).

In summary, results from animal studies suggest Pb exposure is capable of adversely affecting bone growth and density, potentially manifesting its action through interference with growth and hormonal factors as well as toxic effects directly on bone.

5.8.4 Regulation of Bone Cell Function in Animals – Systemic Effects of Lead

Lead may exhibit multiple complex systemic effects that ultimately could influence bone cell function. As discussed in the animal studies below, Pb can modulate alterations in calcium binding proteins and in calcium and phosphorus concentration in the blood stream, in addition to potentially altering bone cell differentiation and function by altering plasma levels of growth hormone and calciotropic hormones such as vitamin D_3 [1,25-$(OH)_2D_3$] and parathyroid hormone.

5.8.4.1 Hypercalcemia/Hyperphosphatemia

Intravenous injection of Pb has been shown to produce both an acute hypercalcemia and hyperphosphatemia in rats (Kato et al., 1977). Injection of a relatively high dose of 30 mg/kg Pb resulted in maximum values of calcium (17 mg%) after one hour and maximum values of phosphorus (13.5 mg%) after 30 minutes. After 12 hours, the levels of both calcium and phosphorus had returned to baseline levels. Histochemical examination demonstrated deposition of Pb into bone and dentin in the rats, suggesting a direct action of Pb on bone and/or teeth, ultimately displacing calcium and phosphorus and thereby producing hypercalcemia and hyperphosphatemia. Blood Pb levels were not reported.

5.8.4.2 Vitamin D [1,25-$(OH)_2D_3$]

As discussed above, vitamin D [1,25-$(OH)_2D_3$] modulates the normal range of calcium in serum. In rats fed a low calcium or low phosphorus diet, ingestion of 0.82% Pb in the diet reduced plasma levels of 1,25-$(OH)_2D_3$; however, this effect is lost when a high calcium or normal phosphorus diet is given (Smith et al., 1981), suggesting a high calcium/phosphorus diet

reduces the susceptibility of vitamin D system to the effect of Pb. No mobilization of calcium from bone or elevation of inorganic phosphorus was seen. Ronis et al. (2001) also reported no effects of Pb on plasma concentrations of vitamin D metabolites, 25-OH D_3 or 1,25-$(OH)_2D_3$, in pubertal male rats exposed to either 0.15% or 0.45% Pb acetate in drinking water and maintained on an adequate diet. High blood Pb levels (over 350 µg/dL) were reported in some animals in both of these studies. Fullmer (1995) found vitamin D function was severely compromised in young growing chicks given a diet low in calcium (0.1% calcium) for two weeks and then exposed to 0.2% or 0.8% Pb in their diet for an additional one or two weeks. In chicks maintained on an adequate diet (1.2% calcium), exposure to 0.2% or 0.8% Pb in the diet resulted in increased plasma levels of 1,25-$(OH)_2D_3$ as well as significantly increased intestinal Calbindin-D protein [a calcium binding protein induced by 1,25-$(OH)_2D_3$] and its associated mRNA, when compared with unexposed control chicks. Levels of intestinal Calbindin-D mRNA and protein and plasma levels of 1,25-$(OH)_2D_3$ were elevated during the first week of Pb exposure to chicks fed a diet deficient in calcium, but were significantly decreased by the second week of Pb exposure. The study suggested Pb was mediating its effect through 1,25-$(OH)_2D_3$, rather than via a direct action on the Calbindin-D protein. Follow up studies by Fullmer et al. (1996) confirmed dose dependent increases in serum 1,25-$(OH)_2D_3$ levels (and Calbindin-D protein and mRNA) with increasing dietary Pb exposure (0.1% to 0.8%) in similar experiments performed on Leghorn cockerel chicks fed an adequate calcium diet. No blood Pb levels were reported in either study.

5.8.4.3 Parathyroid Hormone

At least one animal study has associated experimental Pb exposure with secondary hyperparathyroidism. Szabo et al. (1991) exposed Wistar Kyoto rats to either 1% Pb acetate in water for a short term (10 weeks) or varying concentrations (0.001 to 1% Pb acetate) for a longer term (24 weeks) to assess the influence of Pb on the interaction of the parathyroids with 1,25-$(OH)_2D_3$. Blood Pb levels in the short term experiment were reported simply as less than 0.2 µg/dL in control animals and greater than 50 µg/dL in the Pb-exposed animals. No levels were reported for the longer term experiment. Short term administration of 1% Pb resulted in significant increases in bone Pb; however, total serum calcium and ionized serum calcium were significantly decreased, as compared to controls. Circulating levels of 1,25-$(OH)_2D_3$ were also

decreased, though the rats were maintained on a normal calcium diet (0.95%). Parathyroid glands from rats exposed short term to Pb were significantly increased in size over those in control animals (178 µg per gland versus 96 µg per gland) and specific binding of 1,25-$(OH)_2D_3$ to parathyroid and intestinal tissue was increased. Likewise, long term administration of 1% Pb resulted in significant increases in bone Pb and normalized parathyroid gland weights, and a significant decrease in the level of 1,25-$(OH)_2D_3$. In the long term study, a dose-dependent increase in parathyroid weight occurred with increasing exposure to Pb in drinking water. The authors concluded the secondary hyperparathyroidism was associated with, and/or a result of, the hypocalcemia and decreased 1,25-$(OH)_2D_3$ levels secondary to Pb exposure.

5.8.4.4 Growth Hormone

As discussed in Section 5.8.3, exposure to Pb has been associated with altered bone metabolism and decreased growth and skeletal development (Hamilton and O'Flaherty, 1994, 1995; Gruber et al., 1997; González-Riola et al., 1997; Escribano et al., 1997; Ronis et al., 1998a,b, 2001; Camoratto et al., 1993), suggesting perturbation of one or more endocrine factors such as growth hormone. To examine the effect of exposure to low-level Pb on pituitary growth hormone release, Camoratto et al. (1993) exposed pregnant female rats to 0.02% Pb nitrate (125 ppm Pb) beginning on gestational day 5 and continuing in pups through postnatal day 48. Basal release of growth hormone from control and Pb-exposed pups at age 49 days was not significantly different. Growth hormone releasing factor-stimulated release of growth hormone from pituitaries of Pb-exposed pups was smaller than the stimulated release of growth hormone from pituitaries of control animals (75% increase over baseline versus 171% increase, respectively), but the difference did not achieve significance ($p = 0.08$). Growth hormone content of the pituitary glands was also not influenced by Pb exposure. Ronis et al. (1998b) reported similar findings in rat pups exposed to 0.05%, 0.15%, or 0.45% Pb acetate in drinking water from gestation day 5 through postnatal day 85, with the exception being significantly elevated pituitary growth hormone levels at postnatal day 55. Blood Pb levels for both of these studies were reported above in Section 5.8.3. Taken together, these rat studies suggest that differences in growth seen with Pb exposure may not necessarily be the result of alterations in secretion of growth hormone.

5.8.5 Bone Cell Cultures Utilized to Test the Effects of Lead

5.8.5.1 Bone Organ Culture

In an early bone organ culture study utilizing incorporated radioactive Pb into fetal radii and ulnae, Rosen and Wexler (1977) reported release of Pb as the concentration of calcium in the media was reduced or with addition of parathyroid hormone, but that calcitonin inhibited the release of Pb as expected, verifying the capacity of this model system. The bone organ system was subsequently used to evaluate the efficacy of Pb chelating agents, such as D-Penicillamine and $CaNa_2EDTA$ (Rosen and Markokwitz, 1980; Rosen et al., 1982).

5.8.5.2 Primary Cultures of Osteoclasts and Osteoblasts

The ability to isolate primary cultures of osteoclasts and osteoblasts from mouse calvaria provided an additional experimental model system to study the effects of Pb on specific bone cells. Using isolated osteoclasts and osteoblasts, Rosen (1983) reported that uptake of radioactive Pb by osteoclasts was rapid, almost linear, while osteoblasts showed very little increase in uptake of Pb at increasing media concentrations. Physiological concentrations of parathyroid hormone markedly increased uptake of Pb and calcium by osteoclast cells and, once loaded with Pb, osteoclasts were capable of releasing Pb slowly into the media. Further kinetic analysis of cultured osteoclastic bone cells indicated that cellular Pb is primarily associated with the mitochondrial fraction (~78%) and that this Pb is readily exchangeable with the outside media (Pounds and Rosen, 1986; Rosen and Pounds, 1988). Experiments conducted to characterize the steady-state kinetic distribution and metabolism of calcium and Pb supported the concept that the two elements are metabolized similarly in the osteoclast cells (Rosen and Pounds, 1989).

5.8.5.3 Rat Osteosarcoma Cell Line (ROS 17/2.8)

In recent years, the rat osteosarcoma cell line ROS 17/2.8 has been used extensively to investigate the influence of Pb on various cellular processes and kinetics within these osteoblast-like cells. The ROS 17/2.8 cell model is useful in that the cells are capable of producing osteocalcin (a bone protein important for proper bone mineralization), have high alkaline phosphatase activity (an enzyme normally associated with mineralization of cartilage), possess vitamin D receptors, and respond to parathyroid hormone. In comparisons of cellular Pb

toxicity and metabolism between primary cell culture from mouse calvaria and the rat osteosarcoma cell line, Long and co-workers (1990) reported remarkable similarities in the profile of radiolabeled Pb kinetics and intracellular Pb distribution. Using this cell line, Schanne and co-workers (1989) simultaneously measured intracellular Pb and calcium concentrations and found 5 and 25 micromolar Pb produced sustained 50% and 120% (respectively) increases in intracellular calcium over a 5 hour period, and that measurable entry of Pb into the cells could be demonstrated at the higher concentration. These findings advanced the hypothesis that perturbation of intracellular calcium concentration may be the mechanism of Pb bone toxicity. Schirrmacher and co-workers (1998) reported that calcium homeostasis is upset within 20 minutes of its addition to calvarial bone cell culture. Their results suggested that the calcium-ATPases of intracellular stores were potentially poisoned by Pb entering the cells. Wiemann et al. (1999) demonstrated that Pb was also capable of interfering with the calcium release activated calcium influx (CRAC) in calvarial bone cell cultures. Pb was found to partially inhibit the influx of calcium into the bone cells, plus influx of Pb into the cells was greatly enhanced (2.7 fold) after CRAC had been induced. These effects of Pb were found to be independent of any inhibitory effect on calcium-ATPase.

Miyahara et al. (1995) did a series of experiments in ^{45}Ca-labeled bone organ culture to determine whether the Pb-induced hypercalcemia was the result of the active process of biological bone resorption or simply physiochemical mineral dissolution. Lead introduced into the culture at concentrations of 50 μM and above stimulated the release of calcium and hydroxyproline into the medium; however, no release was elicited from bones inactivated by freezing and thawing. Pb-stimulated ^{45}Ca release was inhibited by bafilomycin A_1, eel calcitonin, and scopadulcic acid B, suggesting the release was secondary to osteoclastic bone resorption. Further evidence to support this conclusion came from experiments examining the influence of two inhibitors of cyclooxygenase on Pb-induced bone resorption. Lead was found to stimulate prostaglandin E_2 release and in cultures, there was a high correlation between prostaglandin E_2 released into the media and ^{45}Ca release. In the presence of cyclooxygenase inhibitors (blocking prostaglandin synthesis), Pb-stimulated ^{45}Ca release was inhibited, suggesting the mechanism of bone resorption in this instance was via a prostaglandin E_2-mediated mechanism.

Lead has been demonstrated to directly impair production of osteocalcin by ROS 17/2.8 cells by 70% after 24 hours of exposure to 25 micromolar Pb (Long et al., 1990). The resulting decrease in cell proliferation is in agreement with similar studies by Sauk et al., 1992). Interestingly, exposure of dental pulp cells, which also produce osteocalcin, to a similar concentration of Pb reduced osteocalcin production by 55% after 12 hours of exposure (Thaweboon et al., 2002). Vitamin D has been shown to increase osteocalcin production in ROS 17/2.8 cells; however, Pb inhibited the vitamin D-stimulated osteocalcin production in a dose-dependent manner from 0 up to 25 micromolar concentrations, plus was shown to be capable of attenuating basal (non-vitamin D-stimulated) osteocalcin production (Long et al., 1990). Lead (5 to 20 micromolar) inhibition of vitamin D stimulation of osteocalcin in ROS cells was also reported by Guity and co-workers (2002). Later studies suggested that Pb acts by inhibiting vitamin D activation of calcium channels and interferes with regulation of calcium metabolism (Schanne et al., 1992), though apparently this effect is not mediated via PKC (Guity et al., 2002). Angle and co-workers (1990) reported that 24 hours of incubation with vitamin D (10 nM) was capable of evoking a 4 to 5 fold increase in osteocalcin production and a 100% increase in cellular alkaline phosphatase activity in ROS cells. Osteocalcin production and cellular DNA contents were increased 100% and 20% respectively by addition of insulin-like growth factor (92.5 ng/mL). Consistent with a toxic effect of Pb on osteoblast function, the addition of 1 to 10 μM Pb to the system inhibited both basal and stimulated osteocalcin secretion, alkaline phosphatase activity and DNA contents (Angle et al., 1990). Dose- and time-dependent reduction in alkaline phosphatase activity with Pb exposure (2 to 200 micromolar) has also been reported in osteosarcoma cells, along with parallel reductions in steady state levels of alkaline phosphatase mRNA levels (Klein and Wiren, 1993). No effect on cell number or DNA and protein synthesis was seen at these levels of Pb exposure.

Though the exact mechanism of Pb toxicity on osteocalcin was unclear, Pb was known to inhibit some of the functional properties of osteocalcin including inhibition of osteocalcin adsorption to hydroxyapatite. An investigation by Dowd and co-workers (1994) utilized the ability of osteocalcin added to a solution of $^{43}CaCl_2$ to broaden ^{43}Ca resonance, as a method to examine binding of calcium to osteocalcin and the influence of Pb on calcium binding. It was determined that the dissociation constant of calcium for osteocalcin was 7 micromolar, while the dissociation constant for Pb was determined by competitive displacement to be 2 nM, indicating

more than three orders of magnitude tighter binding of Pb than calcium to osteocalcin and the likelihood that even submicromolar levels of free Pb would significantly inactivate osteocalcin. Circular dichroism indicated that upon binding, Pb induces a similar structural change in osteocalcin to that found with calcium binding, but the binding with Pb occurs at 2 orders of magnitude lower than with calcium (Dowd et al., 2001). Similarly, hydroxyapatite binding assays indicated Pb causes an increased absorption to hydroxyapatite that is similar to calcium, but again at 2 to 3 orders of magnitude lower concentration, potentially leading to low bone formation rates and/or density (Dowd et al., 2001).

Besides perturbation of calcium metabolism, Pb has been shown to reduce intracellular free magnesium concentrations by 21% in osteosarcoma cells incubated in 10 micromolar Pb for 2 hours (Dowd et al., 1990). Under these same conditions, the unidirectional rate of ATP synthesis (i.e., P_i to ATP) was reduced by a factor greater than 6 over control cultures. Impairment of both of these processes by Pb could ultimately influence bone growth and development.

Lead has also been show to perturb Epidermal Growth Factor's (EGF) control of intracellular calcium metabolism and collagen production in ROS cells (Long and Rosen, 1992). EGF is known to activate protein kinase C (PKC), resulting in increased calcium influx and through this mechanism, decreased collagen synthesis. Incubation of ROS cells with 5 micromolar Pb and 50 ng/mL EGF for 20 hours resulted in a 50% increase in total cell calcium versus the calcium increase seen in cells treated with EGF alone, suggesting more than one site of action is involved in calcium messenger perturbation. A similar finding was reported by Long and co-workers (1992) who found that treatment of Pb (25 micromolar) intoxicated osteosarcoma cells with parathyroid hormone (PTH, 400 mg/mL) resulted in a greater increase in cell calcium than with either treatment alone. Supplementary inhibition of collagen synthesis has also been reported with the addition of 25 micromolar Pb plus 50 ng/mL EGF, suggesting more than one site of action for the effect of Pb on collagen synthesis (Long and Rosen, 1992). Additional study has since suggested that Pb activates PKC in ROS cells and that PKC mediates the rise in intracellular calcium (Schanne et al., 1997). The observation that calphostin C, an inhibitor of PKC, prevented the Pb-induced elevation of intracellular calcium supported this hypothesis, as did the fact that free Pb at concentrations of 10^{-11} to 10^{-7} M directly activated PKC

in the absence of activating concentrations of calcium. This would suggest Pb is capable of activating PKC at concentrations ~3,000 times lower than calcium.

Finally, Pb has been shown to be capable of inhibiting secretion of osteonectin, a bone related protein found in areas of active morphogenesis (Sauk et al., 1992). Treatment of ROS 17/2.8 cells with Pb (4.5×10^{-6} M to 4.5×10^{-7} M) demonstrated that intracellular osteonectin levels were actually enhanced; however, the secretion of osteonectin into the media was delayed or inhibited. Protein production of collagen and the endoplasmic reticulum protein, Hsp47, were relatively unaffected by Pb at these concentrations. The intracellular retention of osteonectin coincided with a decrease in levels of osteonectin mRNA, suggesting the processes associated with translation and secretion of osteonectin are sensitive to Pb.

5.8.5.4 Human Osteosarcoma Cells (HOS TE 85)

Evidence exists that Pb is directly osteotoxic to bone cells in culture. Studies examining the sensitivity of human osteosarcoma cells (HOS TE 85) to Pb found proliferation of the cells was inhibited at Pb concentrations of 4 µmol/l, while cytotoxicity occurred at the 20 µmol/l Pb concentration (Angle et al., 1993). In parallel experiments, rat osteosarcoma cells (ROS 17/2.8) were found to be somewhat less sensitive to the effects of Pb with inhibition of proliferation occurring at 6 µmol/l Pb concentration and cytotoxicity at Pb concentrations over 20 µmol/l.

5.8.5.5 Chick Chondrocytes

The effects of Pb on cartilage biology have been examined in isolated avian chondrocytes obtained from 3 to 5 week old chicks (Hicks et al., 1996). Exposure to media containing 0.1 to 200 µM Pb acetate or chloride were found to decrease thymidine incorporation, suppress alkaline phosphatase, and suppress both type II and type X collagen expression at the mRNA and protein levels. Cytotoxicity of the cultures from Pb exposure was dismissed as proteoglycan synthesis was found to be augmented, suggested Pb selectively inhibits specific aspects of the chondrocyte growth plate. Using the avian chondrocyte model, Zuscik et al. (2002) similarly reported Pb exposure (1 to 30 µM) causing a dose-dependent inhibition of thymidine incorporation into the growth plate, with a 60% reduction in proliferation at the highest concentration. Addition of TGF-β1 and PTHrP, regulators of growth plate, both separately stimulated thymidine incorporation, an effect that was dose-dependently blunted in the presence of Pb. At the highest

Pb concentration (30 μM), inhibition was significantly less in the chondrocytes treated with Pb + TGF-β1 (24%) and Pb + PTHrP (19%) than for Pb alone (60%), suggesting the interaction of Pb with these growth factors may be independent of its primary action on the chondrocyte cells. Support for a direct action of Pb on these growth regulators is supported by the finding that normal TGF-β1 and PTHrP suppression of type X collagen expression is significantly reversed in a dose-dependent fashion in the presence of Pb. This effect evidently was not mediated by BMP-6 (Bone Morphogenic Protein), an inducer of terminal differentiation known to partially reverse the inhibitory effect of PTHrP, because in the presence of Pb, PTHrP significantly suppressed BMP expression, while combined exposure to Pb and TGF-β1 increased BMP expression ~3-fold. Further experiments performed on chick sternal chondrocyte cultures, utilized PTHrP responsive (AP-1) and non-responsive (NF-κB) reporter constructs to examine potential effects of Pb on signaling. While having no effect on the basal activity of the AP-1 reporter, Pb dose-dependently enhanced PTHrP induction of the responsive AP-1 reporter. Lead dose-dependently inhibited the basal activity of the non-PTHrP responsive, NF-κB reporter. Taken together, these studies demonstrate that Pb has an inhibitory effect on the process of endochondral bone formation and that the effects of Pb are likely from its modulation of growth factors and second messengers involved in cell signaling responses.

5.8.6 Bone Lead as a Potential Source of Toxicity in Altered Metabolic Conditions

Lead is avidly taken up by bone and incorporated into bone matrix, where a substantial amount can remain over the lifetime of an organism. The uptake and incorporation of Pb into bone during acute exogenous exposures may be of short term benefit by limiting the exposure of other, more sensitive tissues; however, this does not eliminate Pb from the system. Subsequent release of Pb from this endogenous storage can produce a lifetime of steady, low level Pb exposure during periods of normal bone remodeling, while elevated Pb release during times of increased bone metabolism and turnover (i.e., pregnancy, lactation, menopause, and osteoporosis) can elevate blood levels of Pb significantly, potentially to toxic concentrations. This is especially relevant when there is concurrent exogenous exposure to Pb, as current blood Pb levels are a composite of current and past Pb exposure. Of greater concern is the mobilization of Pb during pregnancy and subsequent transfer to the developing brain of the fetus across the

poorly developed blood:brain barrier. Maternal Pb also appears in breast milk, providing further exposure of the infant to Pb during lactation. Currently, the majority of animal studies examining mobilization of Pb from bone stores have focused principally on elevation of Pb levels or transfer of Pb, rather than reporting toxic effects associated with these exposures. Note that in most instances the mobilization and elimination of Pb is much faster in laboratory animals than in humans. For example, as discussed in Section 5.8.3, Hać and Kruchniak (1996) reported ~64% of Pb given over a 12 week period remained in the bones of rats 64 days post exposure. Therefore, the caveats of experiments performed in small animals, especially when examining mobilization of Pb stores, must be taken into consideration.

5.8.6.1 Pregnancy and Lactation

Pregnancy, and to a much greater extent, lactation, place significant calcium demands on the mother as she provides all the necessary calcium requirements of the developing fetus/infant. During these times of metabolic stress, increased demineralization of maternal bone occurs to supplement demand, unfortunately accompanied by the concurrent mobilization and release of Pb stored in the maternal skeleton from past exposure. Studies in several animal models have shown that maternal bone Pb can be mobilized during pregnancy and lactation, ultimately being transferred to the fetus during gestation and breast feeding. Keller and Doherty (1980) administered radiolabeled Pb drinking water (200µg/mL) to female mice for 105 days prior to mating or 105 days prior to mating and during periods of gestation and lactation (total 160 days of exposure). The results suggested very little Pb was transferred from mother to fetus during gestation, however, Pb transferred in milk and retained by the pups accounted for 3% of the maternal body burden of those mice exposed to Pb prior to mating only. No blood Pb levels were reported for any of the animals. The amount of Pb retained in these pups exceeded that retained in the mothers, suggesting lactation effectively transfers Pb burden from mother to suckling offspring. Transfer of Pb from mothers was significantly higher when Pb was supplied continuously in drinking water, rather than terminated prior to mating. Considerably higher lactational transfer of Pb from rat dams compared to placental transfer has also been reported (Palminger Hallén et al., 1996). Continuous exposure of rat dams to Pb until day 15 of lactation resulted in milk Pb levels 2.5 times higher than in whole blood, while termination of maternal Pb exposure at parturition yielded equivalent blood and milk levels of Pb, principally from Pb

mobilized from maternal bone. Blood Pb concentrations at day 15 of lactation were 1.4 ± 0.4 µg/dL (control), 32.0 ± 5.5 µg/dL (Pb-exposed until parturition), and 126.0 ± 17.1 µg/dL (Pb-exposed until day 15 of lactation).

Using rats chronically exposed to Pb in drinking water, Maldonado-Vega et al. (1996) studied intestinal absorption of Pb, its mobilization, and redistribution during lactation. In rats exposed to Pb 144 days prior to lactation, the process of lactation itself elevated blood Pb and decreased bone Pb, indicating mobilization of Pb from bone as there was no external source of Pb during the lactation process. Rats exposed to Pb for 158 days (144 days prior to lactation and 14 days during lactation) also experienced elevated BLLs and loss of Pb from bone. Lead exposure only during the 14 days of lactation was found to significantly increase intestinal absorption and deposition (17 fold increase) of Pb into bone compared to non-pregnant rats, suggesting enhanced absorption of Pb takes place during lactation. As in other previous studies, the highest concentration of Pb in bone was found in non-pregnant non-lactating control animals, with significantly decreased bone Pb in lactating rats secondary to bone mobilization and transfer via milk to suckling offspring. Blood Pb levels at day 14 of lactation or equivalent ranged from 24.7 to 31.2 µg/dL. Follow-up studies examining the influence of dietary calcium found when calcium was altered from the normal 1% to 0.05%, bone calcium concentration decreased by 15% and bone Pb concentration decreased by 30% during the first 14 days of lactation (Maldonado-Vega et al., 2002). In non-lactating rats on the 0.05% calcium diet, there were also decreases in bone calcium, but neither incremental bone resorption nor Pb efflux from bone, suggesting the efflux from bone during lactation was related to bone resorption. Of interest, enhancement of calcium (2.5%) in the diet of lactating rats increased calcium concentration in bone by 21%, but did not decrease bone resorption, resulting in a 28% decrease in bone Pb concentration and concomitant rise in systemic toxicity. Blood Pb levels were similar to those reported in the prior study above. In both studies, the authors concluded that Pb stored in bone should be considered a major source of self-intoxication and of exposure to suckling offspring.

In one of few studies showing a toxic effect, Han et al. (2000) demonstrated adverse effects in rat offspring born to females whose exposure to Pb ended well before pregnancy. Five week-old-female rats had been given Pb acetate in drinking water (250 mg/mL) for five weeks, followed by a one month period without Pb exposure before mating. To test the influence of dietary calcium on Pb absorption and accumulation, some pregnant rats were fed diets deficient

in calcium (0.1%) while others were maintained on a normal calcium (0.5%) diet. As expected, all Pb-exposed dams and pups had elevated blood Pb levels; however, pups born to dams fed the diet deficient in calcium during pregnancy had higher blood (up to 24 µg/dL) and organ Pb concentrations compared to pups from dams fed the normal diet. Significantly, pups born to Pb-exposed dams had lower mean birth weights and birth lengths than pups born to non-Pb-exposed control dams ($p < 0.0001$), even after confounders such as litter size, pup sex, and dam weight gain were taken into account. The authors concluded that while increases in dietary calcium during pregnancy are capable of reducing Pb accumulation in the fetus, they cannot prevent the decreases in birth weight and length associated with pre-maternal Pb exposure and subsequent mobilization. This has relevance in human pregnancy, as many women experience exposure to Pb during their lifetimes (especially during childhood) and mobilization of the Pb from bone stores during pregnancy could present toxic complications.

Within the last decade, an invaluable method to explore the kinetics of Pb transfer from bone to blood has been developed and evaluated (Inskip et al., 1996; O'Flaherty et al., 1998). The method utilizes recent administration of sequential doses of Pb mixes enriched in stable isotopes (^{204}Pb, ^{206}Pb, and ^{207}Pb) to female cynomolgus monkeys (*Macaca fascicularis*) that have been chronically (1,300 to 1,500 µg Pb/kg body weight per day for ten years or greater) administered a common Pb isotope mix. The stable isotope mixes serve as a marker of recent, exogenous Pb exposure, while the chronically administered common Pb serves as a marker of endogenous (principally bone) Pb. From thermal ionization mass spectrometry analysis of the Pb isotopic ratios of blood and bone biopsies collected at each isotope change, and using end-member unmixing equations, it was determined that administration of the first isotope label allows measurement of the contribution of historic bone stores to blood Pb. Exposure to subsequent isotopic labels allowed measurements of the contribution from historic bone Pb stores and the recently administered enriched isotopes that incorporated into bone (Inskip et al., 1996). In general the contribution from the historic bone Pb (common Pb) to blood Pb level was constant (~20%), accentuated with spikes in total blood Pb due to the current administration of the stable isotopes. Blood Pb ranged from 31.2 to 62.3 µg/100 g in the animals. After cessation of each sequential administration, the concentration of the signature dose rapidly decreased. Initial attempts to apply a single-bone physiologically based model of Pb kinetics were unsuccessful until adequate explanation of these rapid drops in stable isotopes in the blood were

incorporated (O'Flaherty et al., 1998). Once revisions were added to account for rapid turnover of the trabecular bone compartment and slower turnover rates of cortical bone compartment, an acceptable model evolved. From this model it was reported that historic bone Pb from 11 years of continuous exposure contributes ~17% of the blood Pb at Pb concentrations over 50 µg/dL, reinforcing the concept that the length of Pb exposure and the rates of past and current Pb exposures help determine the fractional contribution of bone Pb to total blood Pb levels (O'Flaherty et al., 1998). The turnover rate for cortical (~88% of total bone by volume) bone in the adult cynomolgus monkey was estimated by the model to be ~4.5% per year, while the turnover rate for trabecular bone was estimated to be 33% per year.

Using the method of sequential stable isotope administration, Franklin et al. (1997) examined flux of Pb from maternal bone during pregnancy of 5 female cynomolgus monkeys who had been previously exposed to common Pb (~1,100 to 1,300 µg Pb/kg body weight) for about 14 years. In general, Pb levels in maternal blood (as high as 65 µg/100 g) attributable to Pb from mobilized bone were reported to drop 29 to 56% below prepregnancy baseline levels during the first trimester of pregnancy. This was ascribed to the known increase in maternal fluid volume, specific organ enlargement (e.g., mammary glands, uterus, placenta), and increased metabolic activity that occurs during pregnancy. During the second and third trimesters, when there is a rapid growth in the fetal skeleton and compensatory demand for calcium from the maternal blood, the Pb levels increased up to 44% over pre-pregnancy levels. With the exception of one monkey, blood Pb concentrations in the fetus corresponded to those found in the mothers, both in total Pb concentration and proportion of Pb attributable to each isotopic signature dose (common = 22.1% versus 23.7%, ^{204}Pb = 6.9% versus 7.4%, and ^{206}Pb = 71.0% versus 68.9%, respectively). From 7 to 25% of the Pb found in fetal bone originated from maternal bone, with the balance derived from oral dosing of the mothers with isotope during pregnancy. Of interest, in offspring from a low Pb exposure control monkey (blood Pb <5 µg/100 g) ~39% of Pb found in fetal bone was of maternal origin, suggesting enhanced transfer and retention of Pb under low Pb conditions.

Clearly, the results of these studies show that Pb stored in bone is mobilized during pregnancy and lactation, exposing both mother and fetus/nursing infant to blood/milk Pb levels of potential toxicity. Of equal concern, a significant proportion of Pb transferred from the mother is incorporated into the developing skeletal system of the offspring, where it can serve as

a continuing source of toxic exposure. The above study by Franklin et al. (1997) illustrates the utility of sequentially administered stable isotopes in pregnancy; however, its use may also be applicable in studies of lactation, menopause, osteoporosis, and other disease states where mobilization of bone and release of Pb stores occurs. Furthermore, given that isotopic ratios of common Pbs vary by location and source of exposure, when humans migrate from one area and source of exposure to another, it is possible to document changes in mobilized Pb, especially during times of metabolic stress.

5.8.6.2 Age/Osteoporosis

The age of an animal at the time of exposure to Pb has been shown to influence the uptake and retention of Pb by bone. In experiments to determine the influence of age on this process, Han et al. (1997) exposed rats for five weeks to 250 mg/L Pb acetate in drinking water beginning at 5 weeks of age (young child), 10 weeks of age (mid-adolescence), or 15 weeks of age (young adult), followed by a 4 week period of without Pb exposure. An additional group of rats were exposed to Pb beginning at 5 weeks, but examined following an 8 or 20 week period after cessation of Pb. Significantly lower blood and bone Pb concentrations were associated with greater age at the start of Pb exposure and increased interval since the end of exposure.
No blood Pb levels were greater than ~30 µg/dL. However, young rats beginning exposure to Pb at 5 weeks and examined 20 weeks after cessation of exposure, still had bone Pb concentrations higher than those found in older rats only 4 weeks after cessation of exposure. This showed that exposure to Pb at a young age leads to significant skeletal Pb accumulation and retention, despite the high rate of bone remodeling that occurs during growth and development at that time.

At the opposite end of the spectrum, Cory-Slechta et al. (1989) studied differences in tissue distribution of Pb in adult and old rats. Adult (8 months old) and old (16 months old) rats were exposed to 50 ppm Pb acetate in drinking water for 11 months, at which time the experiment was completed. Bone (femur) Pb levels in older rats were found to be less than those in younger rats; however, blood Pb levels were higher in the older rats. All levels of Pb in the blood were reported to be 31 µg/dL or less. Of interest, brain Pb concentrations in the older rats exposed to Pb were significantly higher, and brain weights were significantly less than the brain Pb concentration and weights of unexposed older control rats or adult rats exposed to Pb, suggesting a potential detrimental effect. The authors suggested that a possibility for the

observed differences in tissue concentrations of Pb was due to changes in the capacity of bone to store Pb with advanced age.

In a subsequent study, Cory-Slechta (1990b) examined kinetic and biochemical responses of young (21 day old), adult (8 months old), and old (16 months old) rats exposed to Pb at 0, 2, or 10 mg Pb acetate/kg/day over a 9.5 month experimental period (blood Pb as high as 45 µg/dL). Results suggested that older rats may have increased vulnerability to Pb due to increased exposure of tissues to Pb and greater sensitivity of the tissues to the effects of Pb. As in the previous study (Cory-Slechta et al., 1989), lower bone Pb levels were present in older rats with concomitant elevated levels of Pb in brain and other tissues, supporting the hypothesis that exposure to Pb over a lifetime may contribute to deterioration of health in old age, potentially during times of heightened bone remodeling such as occurs during osteoporosis.

In studies of bone Pb metabolism in a geriatric, female nonhuman primates exposed to Pb ~10 years previously (historic blood Pb concentration of 44 to 89 µg/dL), McNeill et al. (1997) reported no significant changes in bone Pb level over a 10 month observation period as measured by ^{109}Cd K X-ray fluorescence. The mean half-life of Pb in bone of these animals was found to be 3.0 ± 1.0 years, consistent with data found in humans, while the endogenous exposure level due to mobilized Pb was 0.09 ± 0.02 µg/dL blood. Results examining Pb accumulation in the bones of aging male mice suggest low levels of bone Pb contributing to the osteopenia observed normally in C57BL/6J mice (Massie and Aiello, 1992). The mice were maintained on regular diet (0.258 ppm Pb) and water (5.45 ppb Pb) from 76 to 958 days of age. While the Pb content of femurs increased by 83%, no significant relationship was found between Pb and bone density, bone collagen, or loss of calcium from bone. Blood Pb levels were not reported.

5.8.6.3 Weight Loss

The relationship between body mass and bone mass is highly correlated, and during times of loss of body weight, such as dietary restriction, a concomitant loss of bone mass also occurs. It is therefore possible that Pb stored in bone from prior exposures could be released into the system as skeletal bone is mobilized and result in Pb toxicity. To examine the influence of weight loss on release of stored Pb, Han et al. (1996) first exposed rats to Pb in drinking water (250 mg/l of Pb as acetate) for 5 weeks, followed by a 4 week washout period without Pb to allow primarily accumulation in the skeleton. Rats were then randomly assigned to a weight

maintenance group, a moderate weight loss group (70% of maintenance diet), or a substantial weight loss group (40% of maintenance diet) for a four week period. At the end of this experimental period the blood Pb (24 to 28 µg/dL) and bone Pb levels did not differ between groups, however, the amount and concentration of Pb in the liver increased significantly.
A follow up study in rats previous exposed to Pb for two weeks was undertaken to determine the effect of weight loss and exercise on the distribution of Pb (Han et al., 1999). They found weight loss secondary to dietary restriction to be the critical factor elevating organ Pb levels and, contrary to their first study, elevated blood levels of Pb (as high as 42 µg/dL). No significant difference in organ or blood Pb concentrations was reported between the exercise versus no exercise groups. These studies suggest Pb toxicity could occur in those previously exposed to Pb during times of dietary restriction.

5.8.7 Teeth – Introduction

There was little information in the prior 1986 Lead AQCD relating Pb exposure to adverse outcomes in the teeth of animals. At that time, the incorporation of Pb into teeth was recognized, as was the fact that tooth Pb increased with age, proportional to the rate of exposure and roughly proportional to the blood Pb concentration.

Teeth consist of a hard outer layer of enamel, supported by an underlying layer of dentin, which itself is supported by a connective tissue known as the dental pulp. Enamel is the hardest substance in the body and the most highly mineralized, consisting of ~96% mineral (calcium hydroxyapatite substituted with carbonate ions) and 4% other organic materials, while dentin is only ~70% mineral.

The formation of enamel (amelogenesis) occurs as a two stage process of organic matrix production with ~30% mineralization, followed by removal of water and proteins from the matrix with concurrent further mineralization. As in bone, Pb ions are apparently capable of substituting for calcium ions in the mineralizing tooth, becoming essentially trapped. However, unlike bone, the tooth, with subtle exceptions, does not undergo a remodeling process. Dentin formation (dentinogenesis) can be likened to endochondral bone formation, in that an unmineralized matrix (predentin, rather than cartilage) is laid down first, followed by mineralization to mature dentin. The cells responsible for amelogenesis and dentinogenesis, called ameloblasts and odontoblasts respectively, are similar to osteoblasts in that they respond

to various signaling factors, secrete matrix proteins, and create an environment favorable to deposition of minerals. After enamel formation on a specific tooth is completed, ameloblasts are lost and no additional enamel is laid down with the exception of certain teeth in rodents. These teeth, typically incisors in rats, mice, and most other rodents, continuously erupt to offset the attrition that occurs with daily use. Therefore, the process of amelogenesis is ongoing, albeit confined to a localized area, throughout the life of the animals. For this reason, rodents have been utilized extensively to examine the processes of amelogenesis and the influence of various toxic agents, such as Pb, on tooth development.

Ameloblasts are especially sensitive to toxins and altered metabolic conditions and respond to such insults with disruption of enamel formation. When disruption occurs, defects in the enamel can occur, typically as a band of malformed or altered enamel. As described below, exposure of animals to various concentrations of Pb during tooth development is not only capable of creating distinctive marking of enamel ("Pb lines") but may also influence the resistance of the enamel to dental decay. Within the dental pulp, a layer of odontoblasts continues to reside against the inner layer of the primary dentin for the life of the tooth. During this time, the odontoblasts are systematically slowly putting down thin layers of secondary dentin, slowly decreasing the size of the pulp chamber with age. Lead present during this process has been shown to be readily taken up by this dentin layer, providing a potential marker of historic Pb exposure. Though the enamel is a non-living substance, it is not entirely inert. The external surface of enamel is more or less in a continuous state of flux or turnover as it chemically demineralizes from acids consumed or produced in the mouth by bacteria, followed by remineralization of demineralized enamel when contact with saliva supersaturated with calcium and phosphate ions occurs. Lead present during this process can easily be released from enamel and/or incorporated initially or back into it depending on the circumstances.

In summary, Pb has the potential to disrupt the various processes associated with formation of teeth, plus incorporate itself into all mineralized tooth tissues during formation. Posteruptively, Pb can become incorporated into the secondary dentin, and can be taken up or released from the outer surface layer of enamel during times of remineralization or demineralization. As described below, exposure of animals to Pb has been associated with adverse dental outcomes.

5.8.8 Uptake of Lead by Teeth

As seen with bone, uptake of Pb into the teeth of animals has been demonstrated in a number of studies and by multiple routes of administration. Twenty four hours after a single intraperitoneal injection of radioactive Pb-203 (^{203}Pb, 1 µg/kg) to young (15 day suckling rats) and old (120 day) female rats, 0.7% of the injected dose was present in the four incisor teeth of the younger animals and 0.6% was present in the same teeth of the older animals (Momcilovic and Kostial, 1974). These percentages jumped to 1.43% and 0.88%, respectively, 192 hours after the injection, suggesting incorporation and retention of Pb by teeth is greater in younger animals than in adults, as found in bone. Lead has also been shown to be incorporated into the incisors of rats exposed to airborne Pb. Grobler and co-workers (1991) exposed 6-week-old rats to either "Clean Air" (0.05 µg Pb/m^3) or air containing 77 µg Pb/m^3 and found significant differences in the amount of Pb incorporated into the incisors of the animals. After 70 days, a mean of only 0.8 µg Pb/g of incisor dry mass was found in incisors from control animals, while 11.0 µg Pb/g was present in incisors from the 77 µg Pb/m^3 group. Exposure to air containing 249 µg Pb/m^3 for 28 days or to 1,546 µg Pb/m^3 for 50 days resulted in mean values of 13.8 and 153 µg Pb/g incisor dry weight of Pb incorporation, respectively, highlighting the fact that dose and length of exposure are determinates of amount of Pb contained in the teeth of these animals. Blood Pb levels were 2.6 µg/dL (control), 11.5 µg/dL (low exposure), 24.1 µg/dL (middle exposure), and 61.2 µg/dL (high exposure). Lead has also been shown to be taken up into the teeth of weanling rats whose mothers were exposed to Pb in drinking water. The offspring of pregnant rats exposed during gestation and lactation until 21 days post partum to water containing 0, 3, or 10 ppm Pb showed dose-dependent, significant increases in the Pb content of incisors, first molars, and second molars (Grobler et al., 1985). No blood Pb levels were reported. Taken together, these studies confirm the uptake of Pb into teeth as delivered by various means and suggest that maternal exposure can result in uptake in offspring, during gestation and/or lactation.

5.8.9 Effects of Lead on Enamel and Dentine Formation

Early microscopic studies by Eisenmann and Yaeger (1969) confirmed alterations in rat incisor enamel formation 7 days after a single SC dose of Pb (0.15 or 1.5 mM/100g animal weight); however, no effect was seen at the 0.075 mM/100g dose. Lead was found to have

inhibited mineralization of both enamel and dentin, but only to a "mild to moderate" extent with the mineralization of dentin more affected. It was speculated at the time that Pb could affect the production of normal, mineralizable organic matrix; affect enzymes specific to enamel or dentin formation; affect crystal structure and/or growth; or affect a combination of these factors. In studies of dentinogenesis, incubation of fixed rat molar germs with Pb-pyrophosphate has shown localization of Pb to the mineralization front of dentin (i.e., the area of recently formed dentin), to the stratum intermedium, and to subodontoblastic cells, suggesting Pb may react with mineral components located in the mineralization zone or have a high affinity for these incompletely mineralized areas (Larsson and Helander, 1974). Localization of Pb was also seen at the area of the dentino-enamel junction. Similar examination of first molar germs from 3-day-old rats showed that Pb also localized to the periphery of dentinal globules (Larsson, 1974). A single, high-dose injection of Pb acetate (30 mg/kg body weight) produces an immediate (within 6 h) response in the growing dentin of the rat incisor, leading to the formation of a so-called "Pb line" (Appleton, 1991). A transient rise in serum calcium and phosphorus accompanied the injection, leading to speculation that Pb may have been replacing these minerals in the apatite structure. However, backscattered electron imaging of the Pb line showed it to be composed of continuous hypomineralized interglobular dentin with some incomplete fusion of calcospherites resulting in uneven mineralization, but no localized concentration of Pb was detectable.

This is consistent with Featherstone and co-workers (1981) who reported that Pb incorporation during apatite synthesis was widely dispersed, rather than concentrated in areas of calcium deficiency. Once synthesis is complete, however, Pb is capable of entering calcium deficient areas in enamel, substituting for calcium (Featherstone et al., 1979). This is essentially the process that occurs during demineralization/remineralization of enamel. Appleton (1991, 1992) suggested that Pb has a direct effect on odontoblasts, creating a local disturbance of calcium metabolism, a process similar to that described in bone (Pounds et al., 1991). Interestingly, no ultrastructural changes were seen in ameloblasts from rat pups whose mothers had been drinking water containing Pb.

During the normal process of amelogenesis, water and proteins contained within the organic matrix are lost, leaving densely mineralized enamel. The removal of enamel proteins during this phase is facilitated by enamel proteinases, which are believed to degrade the proteins into smaller units capable of diffusing from the matrix. Using crude extracts from scrapings of

rat incisor teeth, Gerlach and co-workers (2000a) demonstrated that Pb inhibited these proteinases in vitro at micromolar concentrations. In rats given drinking water containing Pb at either 0, 34, or 170 mg/L as Pb acetate for 70 days, increased amounts of proteins were found in enamel matrix from animals exposed to Pb (Gerlach et al., 2002). Moreover, enamel microhardness analysis of upper incisors revealed a significant decrease in microhardness in regions of enamel maturation, but not in areas of fully mature enamel, suggesting Pb exposure mediates a delay in enamel mineralization. In adult rats with incisors trimmed to remove occlusal (biting) contact, a single IP dose of Pb acetate (40 mg/kg) significantly delayed the continuous eruption of the incisor at all time points between 8 and 28 days after dosing, compared with controls (Gerlach et al., 2000b). Blood Pb levels were ~48 µg/dL immediately after injection and 16 µg/dL 30d after injection. It is of interest that delayed eruption of teeth in children living in areas of heavy metal contamination (Pb and zinc) has been reported previously (Curzon and Bibby, 1970).

5.8.10 Effects of Lead on Dental Pulp Cells

Hampered by a general lack of cell cultures specifically for teeth, there remains a paucity of information regarding both the cultures themselves and the effect of Pb upon such cultures. In a single in vitro study using a human dental pulp cell culture obtained from teeth extracted for orthodontic purposes, Thaweboon and co-workers (2002) examined the effects of three concentrations (4.5×10^{-5} M, 4.5×10^{-6} M, 4.5×10^{-7} M) of Pb glutamate on cell proliferation, protein production, and osteocalcin secretion. Under serum free conditions (DMEM only) all concentrations of Pb significantly increased cell proliferation on day 1, day 3 and day 5 of exposure, as measured indirectly by mitochondrial dehydrogenase enzyme assay. In the presence of 2% fetal bovine serum only, the higher concentration of Pb significantly increased protein production, suggesting an influence of serum constituents on cell growth or binding of free Pb in the medium. Similar results were reported when rat osteosarcoma cells (ROS 17/2.8) were exposed to identical concentrations of Pb over 2-, 4-, and 6-day time points (Sauk et al., 1992). Concentrations of Pb less than 4.5×10^{-5} M concentration did not affect osteosarcoma cell proliferation in the presence of serum but, in the absence of serum, 4.5×10^{-7} M Pb increased cell proliferation at day 4, while at day 6, 4.5×10^{-6} M Pb inhibited proliferation. Further testing of human dental pulp cells in serum-free conditions showed that Pb exposure

caused dose-dependent decreases in intracellular protein and procollagen type I production over the 5-day period experimental period (Thaweboon et al., 2002). Short-term exposure of the cells to Pb significantly decreased osteocalcin production in a dose-dependent manner at 8- and 12-h exposure time points. These results suggest that Pb is capable of exerting multiple toxic effects on cells derived from human dental pulp.

5.8.11 Adverse Effects of Lead on Teeth—Dental Caries

In a recent review, Bowen (2001) highlighted 12 epidemiological studies that examined the association between Pb exposure and dental caries (decay), reporting that 8 studies supported the concept that Pb is a caries-promoting element. Unfortunately, the source and actual exposure to Pb and measurement of prevalence of caries varied greatly, providing less than completely satisfactory evidence in the opinion of the author. There is also a paucity of well-controlled animal studies examining this issue.

In an early study examining the effect of drinking solutions containing various metallic ions on dental caries in hamsters, Wisotzky and Hein (1958) reported post-eruptive ingestion of drinking water containing 0.5 mEq of Pb significantly increased caries scores in molar teeth of males after 84 days, but, perplexingly, not in females after 98 days of exposure. It should be noted that in animal studies such as these it is routine to maintain the animals on cariogenic or caries-promoting diets high in fermentable sugars. Clear evidence supporting Pb's role in enhancing susceptibility to dental caries was reported by Watson and co-workers in 1997. In their study, female rats were exposed to Pb in drinking water (34 ppm as Pb acetate) as young adults, during pregnancy, and during lactation. Lead exposure of the subsequent offspring from the dams was, therefore, from transfer of endogenous Pb from dam to pup during gestation and lactation, with no further exposure after weaning. This pre- and perinatal exposure to Pb resulted in a significant, almost 40%, increase in the prevalence of dental caries over control animals. The study was significant for other reasons, as it mimicked the conditions found in many inner cities, where young females are exposed to Pb in their environment and later transfer this Pb to their own fetuses during the extensive bone remodeling that occurs during pregnancy and lactation. The mean blood Pb level in the dams upon weaning was 48 µg/dL, which is not unlike upper levels reported in humans.

The mechanisms by which Pb enhances susceptibility to caries remain uncertain, though clearly altered mineralization and/or incorporation of Pb into enamel as described above could enhance its solubility in acid. Lead also appears in the saliva of rats at about 5% of the whole blood level and at about 61% of the plasma filtrate Pb level (Mobarak and P'an, 1984), providing an avenue for post-eruptive interaction with the exposed enamel in the oral cavity. Notably, decreased salivary flow has been reported in rats exposed to Pb, and decreased salivary function is known to increase caries risk. Stimulated parotid function was decreased by nearly 30% in the Pb-exposed offspring in the study by Watson and co-workers (1997), an effect that could have been mediated by the salivary gland requirement of intact parasympathetic and sympathetic nervous systems for normal development (Schneyer and Hall, 1970) and Pb's known adverse effect on neurotransmitters (Bressler and Goldstein, 1991). Acute infusion of 4 µg of Pb per min was reported to significantly reduce pilocarpine-stimulated salivary secretion in rats over a 50-min period (Craan et al., 1984), whereas 24-day administration of 0.05% Pb acetate significantly reduced the concentration of protein and calcium in pilocarpine-stimulated rat submandibular saliva (Abdollahi et al., 1997). Of potential interest, postnatal exposure of rats to Pb (10 or 25 ppm in drinking water) and a caries-enhancing diet containing fluoride (sucrose containing 15 ppm fluoride) was not associated with an increased risk of dental caries, which suggests that Pb does not interfere with the protective effect of fluoride (Tabchoury et al., 1999). Clearly though, the effect of Pb exposure on salivary gland function and the mechanism by which Pb exposure enhances caries risk needs to be further explored.

5.8.12 Lead from Teeth as a Potential Source of Toxicity

Although no studies currently document the contribution of Pb incorporated into teeth as a source of endogenous Pb exposure, the potential exists during the process of exfoliation of the primary dentition. As described above (Section 5.8.9) Pb is avidly incorporated into the developing dentin and enamel components of teeth. Like bone, the uptake and incorporation of Pb into teeth during acute exogenous exposures may be of short-term benefit by limiting the exposure of other, more sensitive tissues, but, unlike bone, teeth do not undergo a gross remodeling process (the continuous, superficial demineralization/remineralization of the exposed tooth surfaces, principally enamel, are assumed here to be insignificant). However, during the exfoliative process, the erupting secondary tooth erodes away the root (composed of cementum

and dentin) of the overlying primary tooth along with some surrounding alveolar bone. Any Pb incorporated into these portions of bone and primary tooth would be released by the erosive process, with the potential to produce highly elevated local concentrations of Pb in the proximity of remodeling alveolar bone and developing secondary teeth. A more modest contribution to circulating blood Pb would be predicted. Animal research in this area has been hampered, as most common rodents (i.e., rats, mice) are monophyodonts (have only one set of teeth). Although monkeys are an acceptable model, it is problematic as to how release of Pb stored in teeth could be differentiated from that of remodeling skeletal bones formed at a similar time point, plus the disproportionate size of the skeletal mass compared to the dentition may mask any contribution of Pb mobilized by exfoliation.

5.8.13 Summary

- Pb substitutes for calcium and is readily taken up and stored in the bone of experimental animals, potentially allowing bone cell function to be compromised both directly and indirectly by exposure.

- Relatively short term exposure of mature animals to Pb does not result in significant growth suppression, however, chronic exposure to Pb during times of inadequate nutrition have been shown to adversely influence bone growth, including decreased bone density, decreased trabecular bone, and growth plates.

- Exposure of developing animals to Pb during gestation and the immediate postnatal period has clearly been shown to significantly depress early bone growth in a dose-dependent fashion, though this effect is not manifest below a certain threshold.

- Systemically, Pb has been shown to disrupt mineralization of bone during growth, to alter calcium binding proteins, and to increase calcium and phosphorus concentration in the blood stream, in addition to potentially altering bone cell differentiation and function by altering plasma levels of growth hormone and calciotropic hormones such as vitamin D_3 [1,25-$(OH)_2D_3$].

- Bone cell culture studies have indicated that Pb is primarily taken up by osteoclasts and likely perturbs intracellular calcium homeostasis secondary to osteoclastic bone resorption.

- Exposure of bone cell cultures to Pb has been shown to impair vitamin D-stimulated production of osteocalcin, inhibit secretion of bone-related proteins such as osteonectin and collagen, and suppress bone cell proliferation, potentially by interference with such factors as Growth Hormone (GH), Epidermal Growth Factor (EGF), Transforming Growth Factor-Beta 1(TGF-β1), and Parathyroid Hormone-related Protein (PTHrP).

- Periods of extensive bone remodeling, such as occur during weight loss, advanced age, altered metabolic state, and pregnancy and lactation are all associated with mobilization of Pb stores from bone of animals.

- Several animal studies have suggested Pb stored in bone can serve as a continuous, endogenous source of exposure for an individual or can be transferred from mother to offspring during pregnancy and/or lactation, with potentially toxic consequences.

- During pregnancy, transfer of Pb from mother to offspring has been documented, however, available evidence suggests a more significant transfer from mother to offspring occurs during lactation when the concentration of Pb in mother's milk can be several times higher than corresponding blood levels.

- Despite the extensive remodeling of bone that occurs during growth and development of young animals, a significant amount of Pb can be accumulated and retained during times of exposure.

- Pb substitutes for calcium and is readily taken up and incorporated into the developing teeth of experimental animals.

- Unlike bone, teeth do not undergo remodeling per se and, with few exceptions, most Pb incorporated into tooth structure remains essentially in a state of permanent storage.

- Administration of high doses of Pb to animals has demonstrated the formation of a "lead line," visible in both the enamel and dentin and localized to areas of recently formed tooth structure. Within this Pb line, areas of inhibition of mineralization are evident in enamel and dentin.

- Pb has been shown to decrease cell proliferation, intracellular protein, procollagen type I production, and osteocalcin in human dental pulp cells in culture.

- Studies of Pb exposure in adult rats have reported inhibition of post-eruptive enamel proteinases, delayed teeth eruption times, and decreased microhardness of surface enamel.

- During the process of enamel formation, Pb is apparently widely dispersed when first incorporated into the developing apatite crystal; however, post-formation, Pb is capable of entering and concentrating in areas of calcium deficiency within the enamel.

- Numerous epidemiologic studies and, separately, animal studies (both post-eruptive Pb exposure and pre- and perinatal Pb exposure studies) have suggested that Pb is a caries-promoting element; however, whether Pb incorporation into the enamel surface compromises the integrity and resistance of the surface to dissolution and, ultimately increases risk of dental decay, is unclear.

- No animal studies have examined the role exfoliation of the primary dentition in release of Pb previously stored in tooth structure, though it is likely this process could serve as an additional source of Pb exposure in childhood.

5.9 EFFECTS OF LEAD ON THE IMMUNE SYSTEM

5.9.1 Introduction

The immune system, along with the nervous system, has emerged as one of the more sensitive targets of Pb-induced toxicity. However, because Pb exposure at low to moderate levels does not produce overt cytotoxicity of immune cells, immune-associated health effects result from misregulation and shifts in functional capacity rather than profound lymphoid deficiencies. As a result, the most sensitive biomarkers of Pb-induced immunotoxicity are those associated with specific functional capacities as opposed to measures of cell enumeration and/or lymphoid organ pathology. This distinguishes Pb from some other types of immunotoxicants. The following sections provide a survey of the reported immune effects resulting from exposure to Pb in humans and animal models. In general, the focus is on those studies that have been reported since the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986) and have altered our understanding of Pb-induced immunotoxicity.

5.9.2 Host Resistance

Host resistance to disease has been used as an effective measure of the impact of environmental toxicants on immune function. Because different diseases require different combinations of immune effector functions for host protection, analysis of environmental modulation of host resistance across a spectrum of diseases can help identify clinically relevant immunotoxicity.

The 1986 AQCD presented a range of studies in which exposure to Pb inhibited host resistance to disease. Since the time of that report, few new infectious diseases have been added to the list of those that Pb is known to influence. Instead, a much broader understanding of the likely basis for the increased disease susceptibility to these pathogens has become evident. Additionally, recognition of an increased risk for some atopic and autoimmune diseases arising from Pb-induced immunotoxicity has occurred in recent years. This is discussed under Section 5.9.8. Lead-induced alterations of host resistance against infectious and neoplastic diseases are considered in the following sections.

To date, there has been either no effect or an increased susceptibility to disease resulting from exposure to Pb for virtually every infectious agent examined. Given the capacity of Pb to shift immune responses toward Th2, one might expect that enhanced resistance might occur for

diseases where robust Th2 responses were required. For example, an increased resistance against helminth parasitic disease might be hypothesized. However, this possible association has not been widely examined to date.

5.9.2.1 Viral Diseases

In general, exposure to Pb increases the susceptibility to viral infections. Studies include host resistance directed against the encephalomyocarditis virus (Gainer, 1977; Exon et al, 1979), Langat virus (Thind and Khan, 1978), and Semliki Forrest virus (Gupta et al., 2002). In the last example, oral dosing of Swiss mice with Pb acetate (250 mg/kg for 28 days) significantly increased mortality to sublethal doses of the virus. Ewers et al. (1982) reported that occupational exposure to Pb resulted in an increased incidence of influenza cases among workers. In chickens administered Pb acetate orally (20 and 40 mg/100g body weight) for 56 days, antibody production against Newcastle virus vaccine was reduced, while mortality against viral challenge was increased (Youssef, 1996). It seems likely that the reduced Th1 capacity (including effective CTL generation) combined with increased TNF-α, ROI, and prostaglandin E_2 (PGE_2) production by responding macrophages would contribute to increased tissue pathology but reduce viral clearance for many infections.

5.9.2.2 Bacterial Diseases

Most of the Pb-associated host resistance research has been conducted on bacterial diseases. Hemphill et al. (1971) first described the increased susceptibility of mice exposed to Pb (250 µg given i.p. for 30 days) to *Samonella typhimurium*, while Selye et al. (1966) reported increased susceptibility of rats to bacteria endotoxins. Cook et al. (1975) found increased susceptibility of Pb-exposed rats (2 mg/100g body weight given i.v. once) to both *Eschrichia coli* and *Staphylococcus epidermidis.*

The vast majority of studies have been conducted using the intracellular bacterium, *Listeria monocytogenes,* in mice. *Listeria* infection and host resistance to the disease have been well characterized. Essentially, this infection requires an effective antigen presentation (probably involving toll-like receptor 2 involvement), a robust response by activated macrophages leading to interlukin-12 (IL-12) and interferon-γ (IFN-γ) production and robust Th1 driven host protection (Torres et al., 2004; Lara-Tejero and Pamer, 2004). Ideally, activated

macrophages would produce NO in an effective response against *Listeria* (Ito et al., 2005). In the case of Pb-induced immunotoxicity, everything works against this type of response. First, macrophages have severely suppressed NO production. Yet, overproduction of TNF-α, ROIs and PGE_2 leads to tissue inflammation and damage. The skewing of the response toward Th2 means that both IL-12 and IFN-γ are lacking. Excessive production of IL-6 and other pro-inflammatory cytokines results in what has been termed "sickness behavior" which involves both the immune and central nervous systems (Dantzer et al., 1998; Dyatlov et al., 1998a,b; Lawrence and Kim, 2000; Dyatlov and Lawrence, 2002). Lead-induced impairment in host resistance to *Listeria* was reported by Lawrence (1981). CBA/J mice exposed orally to 80 ppm or greater of Pb acetate for 4 weeks had 100% mortality (after 10 days) compared with no mortality for mice exposed to 0 or 16 ppm Pb.

In an important study concerning individual variation in Pb-induced immunotoxicity and host resistance, Kim and Lawrence (2000) demonstrated that neurological circuitry as it pertains to brain-lateralized behavior could impact the effect of Pb on immune responses and host resistance to *Listeria*. Not surprisingly, this suggests that host genotype and epigenetic factors can be influenced by Pb exposure to the individual. Using female BALB/c mice, Kishikawa et al., (1997) demonstrated that exogenously administered recombinant IL-12 (1 μg each for three days i.p.) could enhance production of IFN-γ as well as host resistance to *Listeria* in Pb-exposed (2 mM in water for 3 weeks) mice. However, Pb-exposed mice continued to have excess IL-6 production (part of the sickness behavior phenotype). The result with IL-12 validates the importance of the Th skewing and macrophage impairment induced by Pb on host resistance to certain diseases.

Additional bacterial infections in which Pb exposure has been reported to reduce host resistance include *Serratia marcesens* (Schlipkopter and Frieler, 1979) and *Pasteurella multocida* (Bouley et al., 1977).

5.9.2.3 Parasitic Diseases

Few studies have been conducted to date regarding the effects of Pb on host resistance to parasitic diseases. This is unfortunate as some parasitic disease challenges require effective Th2 responses for optimal resistance. Hence, it is not clear that Pb exposure would depress host resistance in every case (e.g., for helminth parasites). Since the 1986 Lead AQCD, one study

was conducted examining the effect of Pb on the killing ability of *Leishmania enriettii* parasites in vitro by mouse macrophages (Mauël et al., 1989). The authors found that 30 to 100 mM of Pb acetate interfered with the killing ability of macrophages without producing macrophage cytotoxicity.

5.9.2.4 Tumors

The primary study concerning tumor immunity/tumor growth and Pb was already known at the time of the 1986 AQCD. In this study, male C57Bl/6 mice were exposed to Pb acetate in the drinking water at concentrations of 0, 13, 130, or 1300 ppm. Moloney sarcoma virus (MSV)-induced tumor formation and growth were compared following the exposure of mice to Pb for 10 to 12 weeks. MSV-induced transplantable tumors were also used in this study. Primary tumor growth was enhanced in animals that received 130 and 1300 ppm of Pb versus the control. Still, all tumors regressed eventually. Most other studies involving Pb exposure and tumors describe the fact that Pb can exacerbate the ability of other toxins to promote tumor formation (Kobayashi and Okamoto, 1974; Hinton et al., 1979). Much of the tumor-promoting activity of Pb would seem to involve depressed Th1 and macrophage function, as well as the promotion of excessive ROI release into tissues.

5.9.3 Humoral Immunity

The irony of Pb as an immunotoxicant is that the overall effects on humoral immunity are reasonably modest compared to those reported for macrophages and T lymphocytes (McCabe 1994). McCabe et al. (1991) discussed the fact that Pb is not profoundly cytotoxic for most immune cells yet can cause major functional shifts within the immune system as well as decreased host resistance to disease. In many cases, antibody production can remain robust in Pb-exposed animals and humans. However, the nature and spectrum of the antibodies produced is the more significant cause for concern. Lead appears to alter the course of T lymphocyte-driven B cell maturation such that class switching may be skewed in Pb-exposed animals and humans. If Pb dosage and duration of exposure is sufficient, antibody production may be depressed overall. However, with low-level Pb exposure, skewed isotype production is the greater health risk.

5.9.3.1 General Effects on B lymphocytes and Immunoglobulins

Despite the fact that T lymphocytes and macrophages appear to be the more sensitive targets of Pb, the metal can alter B lymphocyte maturation and shift immunoglobulin production. The 1986 AQCD describes the fact that some early studies reported no effect of Pb on antibody production (Reigart and Graber, 1976; Ewers et al., 1982), while others reported a significant decrease in the humoral immune response (Koller, 1973; Koller and Kovaic, 1974; Blakley et al., 1980). In retrospect, this apparent discrepancy may have been caused by the various different Pb concentrations administered, as well as by variations in the duration of exposure. Also, as mentioned in the 1986 AQCD, the temporal relationship of Pb exposure to antigen challenge may be important.

In studies measuring generation of plaque forming cells (PFCs) against sheep red blood cells (SRBCs), Pb incubation with lymphocytes in vitro caused an increased response (Lawrence, 1981b). In a comprehensive study using several strains of mice, Mudzinski et al. (1986) reported that Pb acetate administered in the drinking water (10 mM for 8 weeks) elevated the response in the one strain (BALB/c mice) but failed to alter the humoral response to SRBCs (either PFCs or antibody titers) in all other strains. McCabe and Lawrence (1990) reported that Pb caused an elevation in B cell expression of Class II molecules, thereby influencing B cell differentiation. Lead seemed to impact Class II molecule density at the cell surface via the levels of mRNA translational and/or the posttranslational stages of cell surface protein synthesis (McCabe et al., 1991).

Some human epidemiological and occupational studies have reported Pb-associated differences in levels of circulating immunoglobulins. However, Tryphonas (2001) discussed the pitfalls of relying on total serum immunoglobulin in assessing immunotoxic effects in humans. Sun et al. (2003) reported that immunoglobulin M (IgM) and immunoglobulin G (IgG) were lower but that IgE was higher among females within their high-Pb group. Basaran and Ündeger (2000) found that IgM, IgG, and some complement proteins were reduced among battery workers with high Pb exposure. Results of Ündeger et al. (1996) were similar as well.
In contrast, Sarasua et al. (2000) reported an elevation in immunoglobulin A (IgA), IgG, and IgM associated with environmental Pb exposure. Pinkerton et al. (1998) found no major effects but reported a significant Pb-associated decline in serum IgG and an elevation in B cell

percentage. In a human in vitro study, Borella and Giardino (1991) showed that Pb exposure caused an increased IgG production following stimulation of cells with pokeweed mitogen.

In more recent animal studies, Miller et al. (1998) and Chen et al. (1999) reported no effect on antigen-specific IgG titers against keyhole limpet hemocyanin (KLH) protein in F344 strain rats that had been exposed in utero to Pb (0–500 ppm Pb acetate in drinking water).

It seems likely that Pb exposure may be capable of reducing serum immunoglobulin levels given sufficient dose and duration of exposure. However, the more critical issue pertains to the distribution of class and subclass of immunoglobulins produced after Pb exposure. Because Pb can alter the development of T cells involved in specific antigen responses, this can impact the spectrum of immunoglobulins produced in response to T-dependent antigens. As discussed in the following section, production of IgE (a class of immunoglobulin that is poorly represented in serum but of great clinical significance) is a central issue for Pb-induced immunotoxicity. One additional health concern is the potential for Pb to enhance the likelihood of autoantibody production (Lawrence and McCabe, 2002; Hudson et al., 2003). This latter concern is discussed under Section 5.9.8.

5.9.3.2 IgE Alterations

One of the three predominant hallmarks of Pb-induced immunotoxicity is an increase in IgE production. This can occur in the context of antigen-specific responses or as measured by total serum IgE. For this endpoint, the human and animal findings are very similar. Virtually all of the information concerning the capacity of Pb to elevate IgE production in humans and animals has been obtained since the 1986 AQCD. As a result, this represents a relatively new biomarker for Pb-induced immunomodulation, and one not included in most animal or human studies conducted prior to 1990 (e.g., Wagerová et al., 1986).

Table 5-6 lists the studies reporting Pb-induced elevation of IgE. The disease implications of Pb-induced increases in IgE production are potentially significant and may help to address, in part, the allergy epidemic that has occurred in the last several decades (Isolauri et al., 2004). A relationship has been established between relative Th2 cytokine levels, serum IgE levels, and the risk of allergic airway inflammation (Maezawa et al., 2004; Cardinale et al., 2005). In fact, attempts to manage allergic inflammation use IgE as one of the major targets (Stokes and Casale, 2004). IgE levels are directly related to the production of Th2 cytokines

Table 5-6. Recent Studies Reporting Lead-Induced Increase in IgE

Species	Strain/Gender	Age	In vivo Ex vivo	Lowest Effective Dose	Exposure Duration	Reference
Human	Both genders	Children	Yes	Not available	Not Available	Karmaus et al. (2005)
Human	Both genders, 91% males	Adult	Yes	Not Available	Not Available	Heo et al. (2004)
Human	Females	Children	Yes	Not Available	Not Available	Sun et al. (2003)
Mouse	Balb/c males and females	Fetal	Yes	0.1 mM	3 days	Snyder et al. (2000)
Human	Both genders, 56% male	Juvenile	Yes	Not Available	Not Available	Lutz et al. (1999)
Rat	F344 females	Embryo – fetal	Yes	100 ppm	5 weeks to dam (2 and 3 gestational)	Miller et al. (1998)
Mouse	Balb/c females	Adult	Yes	50 μg 3x per week s.c.	3 weeks	Heo et al. (1996)
Human	Males	Adult	Yes	Not Available	Not Available	Horiguchi et al. (1992)

such as interlukin-4 (IL-4), among others (Tepper et al., 1990; Burstein et al., 1991; Carballido et al., 1995; Takeno et al., 2004; Wood et al., 2004). The relationship between Th2 cytokines (e.g., IL-4), IgE levels, and allergic airway disease is supported through various pharmacological interventions in both animals and humans that either induce Th2 cytokine and promote allergic airway disease (Wu et al., 2004) or interfere with Th2 cytokine-driven IgE production and inhibit allergic inflammation (Holgate et al., 2005; Ban and Hettich, 2005). The production of IgE is of importance in terms of potential inflammation. Not only is the level of IgE a consideration, but also the expression of the Fc receptor for the epsilon (ε) chain of IgE on mast cells and basophils.

In humans, Karmaus et al. (2005) reported a positive association of blood Pb levels with serum IgE concentration among second grade children living near a waste incinerator or other lead-emitting industries. Sun et al. (2003) also found a positive association of blood Pb and serum IgE levels among children in Taiwan. Lutz et al. (1999) reported a correlation of blood Pb levels and serum IgE levels in children in Missouri from 9 months–6 years of age. This association appears to hold not only for children but also for adults. Heo et al. (2004) recently showed that battery workers with blood Pb >30 μg/dL differed significantly in serum IgE levels

from those with blood Pb <30 μg/dL. Additionally, serum IgE concentration correlated with blood Pb among the populations examined (r = 0.0872).

Animal data support this relationship between blood Pb concentration and IgE level and further suggest that even very low-level Pb exposure early in development may produce elevated IgE production in the juvenile offspring. Miller et al. (1998) found that gestational exposure of rats to 100 ppm Pb acetate in the drinking water could produce elevated IgE in the adult offspring. Snyder et al. (2000) showed that gestational and/or neonatal exposure of mice to Pb acetate produced neonatal blood Pb levels that were not above background (5.0 μg/dL) but still could result in elevated IgE production in the juvenile mouse. In most cases, Pb exposures associated with elevated IgE were also associated with increases in IL-4 production by T cells (Chen et al., 1999; Snyder et al., 2000). This is consistent with the fact that high IL-4 production can predispose B lymphocytes to undergo a specific class switch for the production of IgE.

For NK cells, activation can occur through various pathogenic components such as double stranded RNA. However, recently Borg et al. (2004) showed that mature dendritic cells produced a Th1-promoting cytokine, IL-12, and this in turn activates NK cells to produce the further Th1-promoting cytokine, IFN-γ. Interleukin-18 (IL-18) produced by macrophages is also an activator of NK cells, facilitating Th1-promoting cytokine release while interleukin-2 and interleukin-15 (IL-2, IL-15) are growth factors for NK cells. NK cells would appear to be relatively resistant to the effects of Pb compared to some T lymphocytes and macrophages. For a detailed consideration of the effects of Pb on NK cells, see Section 5.9.7.

Cytotoxic T lymphocytes are generated in response to antigen presentation delivered with Th1 cytokines. These cells are capable of mediating antigen specific destruction of neoplastic and virally infected cells via binding and release of cytolytic proteins into the intracellular space. Frequently, the most effective antigen targets of CTLs are the early viral proteins produced in the first phase of host cell infection by viruses. IL-12, produced largely by dendritic cells, appears to be important in the generation of antigen CTL cells and IFN-γ produced by Th1 lymphocytes. NK cells are a potent regulator of CTL activity. Cell signaling via certain toll-like receptors on antigen presenting cells seems to have a role in determining the nature of the Th activation (Th1 versus Th2) and can, therefore, influence the extent of CTL production.

Because T lymphocytes and their regulator and effector functions are so critical in CMI, the maturation of thymocytes within the thymus microenvironment and the selection of

repertoire among the maturing T lymphocytes are crucial issues for potential developmental immunotoxicants. In fact, Pb seems to be capable of disrupting several aspects of T cell maturation, activation, and repertoire usage (McCabe and Lawrence, 1991; Heo et al., 1998; Miller et al., 1998; McCabe et al., 2001, Lee and Dietert, 2003).

5.9.4 General Effects on Thymocytes and T lymphocytes

In general, cells of the T cell lineage appear to be relatively sensitive to the toxic effects of Pb compared to other lymphoid populations. At the time of the 1986 Lead AQCD, there was some understanding of this sensitivity. However, there appear to be considerable differences in sensitivity across various T cell subpopulations (McCabe and Lawrence, 1991; Heo et al., 1996; 1997; 1998). This was largely unknown when the 1986 AQCD was prepared, as the partitioning of T helper cells into functionally distinct subpopulations (e.g., Th0, Th1, and Th2) was not known until the latter part of the 1980s. The differential impact of Pb on T helper cell populations and on immune balance was established during the 1990s. This has become one of the hallmarks of Pb-induced immunotoxicity.

Original observations of both in vivo and in vitro T-dependent immune responses in the presence of Pb suggest that T helper function, as well as the spectrum of cytokines produced, are skewed toward the Th2. The cytokine skewing is discussed as well in Section 5.9.5.3. Smith and Lawrence (1988) have shown that Pb can inhibit antigen presentation and stimulation of a T cell clone of the Th1 phenotype. McCabe and Lawrence (1991) were the first to show that this was caused by the novel capacity of Pb to inhibit Th1 stimulation while promoting presentation to Th2 clones. Heo et al. (1996) provided both in vitro and in vivo results supporting this Pb immunomodulation. Cytokine skewing accompanied the differential stimulation of Th cells.

Using naïve splenic CD4+ T cells derived from D11.10 ovalbumin-transgenic mice, Heo et al. (1998) developed T cell clones in vitro in the presence of Pb. The authors found the T cells that developed from the naïve precursors were significantly skewed toward the Th2 helper phenotype and away from the Th1 phenotype. If IL-4 was inhibited with the addition of anti-IL-4 to the cultures or if the Th1- promoting cytokine IL-12 was added exogenously to the culture, the effects of Pb could be largely overcome. This study provided firm evidence that Pb can directly promote Th2 development among precursor Th(0) cells and impair development of Th1 cells. Among its effects, Pb enhanced adenyl cyclase activity and increased the levels of

cAMP. The authors suggested that Pb may influence cell signaling in such as manner as to promote the Th2 pathway.

Beyond the biasing of immune responses at the level of the T lymphocyte based on Th1/Th2 balance, Pb has the capacity to bias usage of certain Vβ genes (Vβ5, Vβ7, and Vβ13) among T lymphocyte clones in mice (Heo et al., 1997). This is of concern, as it suggests that exposure to Pb may alter the T cell repertoire and skew its representation. Heo et al. (1997) discussed the fact that many autoimmune diseases are characterized by a disproportionate usage of certain Vβ genes. Different autoimmune conditions are associated with the differential overabundant usage of a specific Vβ gene. They suggest that this feature of Pb-induced T lymphocyte immunotoxicity may contribute to and enhance the risk of autoimmunity.

Lee and Dietert (2003) exposed the developing thymus of embryonic day 12 (E12) chickens to Pb acetate (single injection of 400 μg) and evaluated the capacity of thymocytes (ex vivo) from juvenile chickens to produce IFN-γ. They found that embryonic exposure at doses that impair juvenile delayed type hypersensitivity (DTH) also inhibit IFN-γ production. Similarly, IFN-γ production was decreased when thymocytes from juvenile chickens were exposed to Pb in vitro (0.45 μM). However, in vitro exposure of thymic stroma to Pb did not result in suppression of control thymocyte IFN-γ production in co-cultures. There is a suggestion that the balance of reproductive hormones in early life may influence the impact of Pb on developing thymocytes (Hussain et al., 2005).

5.9.4.1 Delayed Type Hypersensitivity

The DTH assay is an in vivo assay requiring antigen-specific T lymphocytes to be primed, expanded, and then recruited to a local site of antigen deposition. The most common application of the DTH is the tuberculin assay for TB in humans. The assay has a long history of application in immunotoxicology, and its utility within the national toxicology program assessment in the mouse has been previously reported (Luster et al., 1992). The assay is known to depend largely on Th1 participation and is, therefore, an effective measure of Th1-dependent function. However, there are at least two different portions of the response that are under somewhat separate control. Priming and expansion of the antigen-specific T lymphocytes is largely Th1 dependent. However, recruitment of T lymphocytes to the periphery involves a variety of locally produced chemotactic signals that may not be under the same regulation. In fact, Chen et al.

(1999) showed that a commonly used chelator for Pb poisoning (succimer, meso-2, 3-dimercaptosuccinic acid [DMSA]) fails to restore Pb-induced suppression of DTH in rats, because the chelator itself somehow interferes with the production of chemotactic factors necessary for T lymphocyte recruitment. The DTH assay is also generally useful in questions of possible developmental immunotoxicity, because of the natural skewing toward Th2 that occurs during gestation through birth and the issue of effective Th1 functional acquisition in the newborn.

Lead-induced suppression of the DTH response is another hallmark of Pb-induced immunotoxicity. At the time of the 1986 AQCD, the capacity of Pb to suppress DTH function was already known from two studies conducted during the late 1970s. However, the association of the function with Th1 help had not been established. Muller et al. (1977) were among the first to demonstrate Pb-induced suppression of DTH. Using mice, these investigators administered Pb acetate i.p. for 30 days prior to assessment of primary and secondary DTH responses against SRBCs. Both primary and secondary responses were severely depressed following exposure to Pb, even at the lowest dose tested (0.025 mg). Faith et al. (1979) exposed developing Sprague-Dawley rats to Pb acetate in the drinking water (lowest dose at 25 ppm) first via the dams during gestation and through weaning and then with direct exposure of the offspring until 6 weeks of age. In this case, the purified protein derivative (PPD) of tuberculin was used as the antigen compared against the saline injection control. Rats administered the lowest dose of Pb evaluated (producing a BLL of 29.3 µg/dL) had a significantly reduced DTH response. Laschi-Loquerie et al. (1984) measured the contact hypersensitivity reaction against picryl chloride in mice that had received 0.5 mg/Kg Pb via s.c. administration. Lead administration was given from 3-6 days in duration at varying times relative to the sensitization period. These investigators reported that Pb suppressed the DTH type of response regardless of the window (before or during sensitization) in which it had been administered.

More recently, Miller et al. (1998) found that female F344 rats gestationally exposed to 250 ppm of Pb acetate in drinking water had a persistently reduced DTH reaction against KLH protein. Chen et al. (1999), Bunn et al. (2001a,b,c) and Chen et al. (2004) had similar findings in studies that included both the F344 and CD strains of rats. In the last study conducted in F344 rats, a BLL of 6.75 µg/dL at 4 weeks of age, postgestational exposure to Pb acetate (250 ppm in drinking water) was associated with depressed DTH against KLH in the 13-week-old adult

female offspring (Chen et al., 2004). McCabe et al. (1999) were among the first to draw attention to the relationship between Pb-induced suppression of DTH and the prior observations of Pb-induced Th skewing. These authors gave varying doses of Pb acetate in drinking water (32,128, 512, 2048 ppm) to female BALB/c mice for 3 weeks prior to measuring the DTH against SRBCs. They found that the 512 ppm dose producing a BLL of 87 µg/dL significantly impaired the DTH response. Antigen routes proved to be important as Pb depressed DTH when an i.v. primed with SRBCs was used, but not when SRBCs were administered i.p. Timing of Pb administration was found to be important relative to the capacity to depress the DTH response. Lee et al. (2001) showed that Pb acetate (200 µg) administered in ovo to chicken embryos at 9 days of incubation failed to depress juvenile DTH against bovine serum albumin (BSA), but when the same dose of Pb was administered 3 days later producing the same BLL, juvenile DTH was severely reduced. Using the latter model, embryonic administration of exogenous thymulin was found to partially restore juvenile DTH function following embryonic exposure to Pb (Lee and Dietert, 2003).

With regard to developmental sensitivity of the DTH response to Pb-induced immunosuppression, parallel findings were obtained in the developing rat (CD strain females) (Bunn et al., 2001c) in agreement with those found in the chicken. Administration of 500 ppm Pb acetate during gestational days 3 to 9 or 15 to 21 produced no DTH effect compared with DTH suppression in the corresponding adult offspring. As shown in Figure 5-17, the sensitivity of the DTH response to Pb appears to develop sometime between days 9 and 15 of rat embryonic development. Apparently, the status of the developing thymus may be a consideration in the capacity of Pb to impact the subsequent DTH response, as discussed further in Section 5.9.10.

It should be noted that in several studies, Pb-induced suppression of the DTH response was associated with reduced capacity to produce the Th1 cytokine, IFN-γ (Chen et al., 1999; Lee et al., 2001).

5.9.4.2 Other T-Dependent Cell-Mediated Immune Changes

The in vitro response of T lymphocyte populations to various mitogens (e.g., Concavavalin A [ConA], Phytohemagglutinin A [PHA]) has been used as a surrogate measure of antigen-driven T lymphocyte stimulation. The impact of Pb on these parameters is presented in Section 5.9.5. Another T cell response altered by exposure to Pb is the mixed lymphocyte

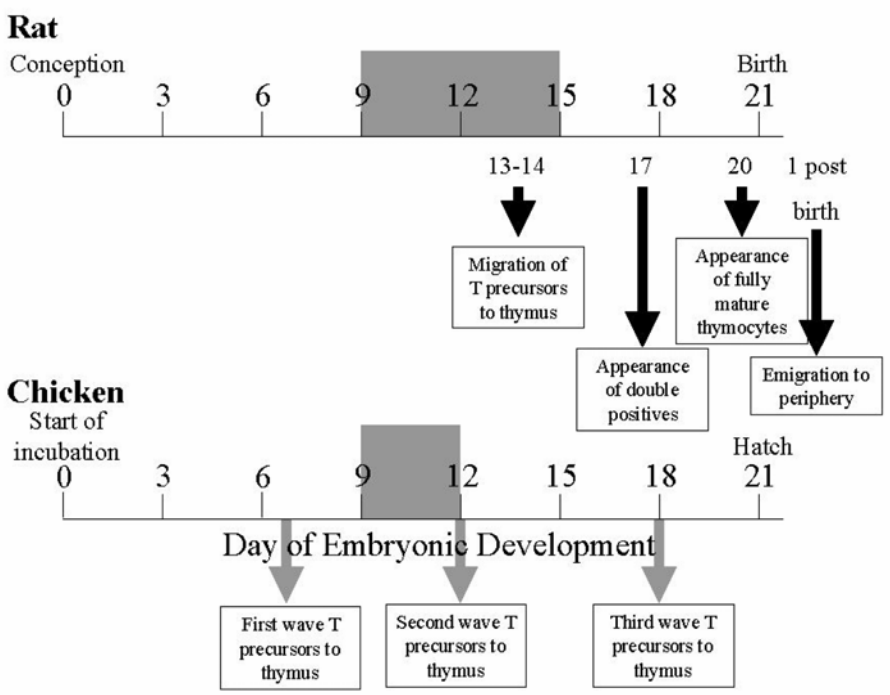

Figure 5-17. Windows during prenatal development (days postconception for rat) or embryonic development (days postincubation initiation for chicken) during which sensitivity of DTH to lead emerges.

response (MLR). This in vitro assay is a measure for the responsiveness of T cells to the presentation of allogeneic major histocompatibility complex (MHC) molecules by antigen presenting cells. The in vivo correlate of the MLR is usually considered to be the graft versus host (GvH) reaction. Several investigators have reported Pb alteration of the MLR as summarized in Table AX5-9.4.

McCabe et al. (2001) demonstrated that Pb at very low physiological concentrations (0.1 μM or approximately the equivalent of 10 μg/dL) in vitro significantly enhanced the proliferation and expansion of murine alloreactive CD4+ T lymphocytes in the MLR reaction.

In fact, the resulting population was found to have a high density of CD4 molecules on the cell surface, making them phenotypically similar to memory T lymphocytes. The authors hypothesized that Pb-induced creation of an exaggerated pool of memory-type T lymphocytes (possessing a lower threshold required for subsequent activation) would be problematic for the host. In a study using Lewis strain rats, Razani-Boroujerdi et al. (1999) also found evidence for Pb-induced stimulation of the in vitro MLR response. In this case, both the alloreactive mixtures

of cells as well as syngeneic mixtures were elevated in proliferation when cultured in the presence of Pb acetate (e.g., 50 ppm or approximately 131 μM). When concentrations of Pb were significantly higher (200 ppm or greater), proliferation was inhibited in these cultures.

Figure 5-17 illustrates the developmental appearance of initial sensitivity for Pb-induced suppression of the DTH function. The mid-embryonic developmental window is the time during which the capacity of Pb to impair later-life DTH responses first emerges. Earlier pulsed exposure to Pb fails to impair juvenile and/or adult DTH despite the continuing of presence of Pb in the embryo. However, during the second half of embryonic development the embryo becomes remarkably sensitive to Pb-induced suppression of DTH. Both the rat and chicken are similar in this window of emerging Th1-dependent functional sensitivity. Thymus-related developmental events are indicated along with the emergence of DTH functional sensitivity to Pb. Information was derived from Gobel (1996), Vicente et al. (1998), Dunon et al. (1998), Dietert et al., (2000), Bunn et al. (2001c), Lee et al. (2001) and Holsapple et al. (2003).

5.9.5 Lymphocyte Activation and Responses

Many of the broader functional ramifications of Pb exposure on lymphocytes are discussed under Sections 5-9.3 and 5-9.4. However, the capacity of Pb to directly alter lymphoid responses is a significant component of Pb-induced immunotoxicity and is summarized within the present section. Lymphoid responses are usually assessed in terms of proliferation and activation (functional changes). One of the recent endpoints reflecting functional status is the production of cytokines. These both autoregulate the producing cells and significantly impact the activity of other immune and nonimmune cells carrying the appropriate receptors. The spectrum and levels of cytokines produced by a population of immune cells tends to reflect their capacity to regulate the host immune response.

5.9.5.1 Activation by Mitogens

The capacity of certain plant- and bacterially derived products to stimulate lymphoid populations to enter the cell cycle and undergo mitogenesis has been used for decades to assess the potential capacity of lymphocytes to receive proliferation signals and expand their population. Among the mitogens employed within the Pb exposure studies are the T lymphocyte subpopulation mitogens, PHA and Con A; the dual T and B cell mitogen, pokeweed mitogen

(PWM); the B lymphocyte mitogen derived from gram-negative bacteria, lipopolysaccharide (LPS), and the B cell mitogen, *Staphylococcus aureus* enterotoxin (SE). It should be noted that these mitogens do not necessarily stimulate all T lymphocytes or all lymphocytes but, instead, stimulate selected populations of the cells. The mitogens react with a large array of cell surface molecules producing cross-linking and appropriate signal transduction to initiate mitogenesis. In the case of the plant-derived mitogens, lectins, numerous glycoproteins and glycolipids carrying the correct carbohydrate residues serve as the cell surface binding sites for cross-linking. Mitogen stimulation in vitro has been used as a surrogate for antigen-driven stimulation and proliferation of antigen-specific T and B cell clones. However, it should be noted that while the assays have been used for decades, there are now more specific assays utilizing more functionally relevant cell surface receptors to assess lymphoid activation potential.

The 1986 AQCD has an extensive review of mitogenic responses of lymphocytes following both in vivo and in vitro treatment by Pb. The results at that time showed no clear pattern. At low to moderate levels, Pb was potentially co-mitogenic for some cells and at very high concentrations could suppress proliferation. Little has changed in conclusions for this assessment measure since the 1986 AQCD. The most significant findings from the mitogenic studies are that at doses encountered physiologically Pb is not a potent cytotoxic agent for most immune cells. At low concentrations, it can marginally stimulate lymphoid mitogenesis. However, as one examines more refined subpopulations of lymphocytes than what were able to be identified prior to 1986 (e.g., Th1 versus Th2 clone of T lymphocytes), it becomes clear that Pb can promote expansion of some lymphoid populations while suppressing others.

Annex Table AX5-9.5 for this section summarizes results of Pb effects on mitogen-stimulated proliferation of lymphoid populations.

5.9.5.2 Activation via Other Receptors

In recent years, lymphoid activation and population expansion has been measured using the triggering of specific T and B cell surface receptors (e.g., CD3 on T cells) as well as antigen-driven proliferation of T cell clones known to be specific for the antigen in question. The latter has provided the opportunity to simulate in vivo lymphoid activation and antigen-driven proliferation by using receptors in vitro, which are more physiologically relevant than those activated by plant lectins. Because Pb does not cause profound population loss across the

entire population of T or B lymphocytes, these more refined and functionally-relevant assay systems have enabled a much clearer picture to emerge concerning Pb-induced changes in lymphoid population than was available for the 1986 AQCD report.

Smith and Lawrence (1988) and McCabe and Lawrence (1991) utilized antigen-specific mouse T clones. They found that Pb directly promoted antigen presentation and stimulation of the T cell clones when these clones were Th2 cells. However, when the Th1 clones were used, Pb suppressed the antigen-specific presentation signal. In the McCabe and Lawrence study, direct comparisons were made between Th1 and Th2 clones specific for mouse allogeneic MHC molecules. These studies provided the first clear picture of the differential effects of Pb on Th1 versus Th2 cells. Several studies since these have verified this major effect of Pb (Heo et al., 1996; 1997, 1998). Many of these later studies utilized the transgenic mouse strain (DO11.10 OVA-tg) that carries T cells specific for a peptide fragment of ovalbumin. These enabled the same comparisons to be made with the presentation of a soluble protein antigen as the stimulating signal. Heo et al. (1998) showed that Pb not only selectively stimulates Th2 cells and suppresses Th1 cells but that it preferentially causes precursor Th0 cells to mature into Th2, rather Th1 cells, as well. Additionally, the T cell clones in the presence of Pb are skewed in terms of their usage of Vβ genes (as reflected in their cell surface receptors) (Heo et al., 1997). This is of particular concern relative to the risk of autoimmunity. More recently, McCabe et al. (2001) examined Pb exposure in the context of the allogeneic MLR against allogeneic MHC molecules. In vitro exposure to Pb (as low as 1.0 μM) enhanced the primary MLR response, but not the secondary MLR response and not the mitogenic response using PHA. Significantly, the T cell clones that emerged from the primary MLR were in greater proportion than normal and were of the specialized phenotype CD4-plus high density ($CD4+^{high}$). Because these fit the phenotype of memory cells, it is likely that an overabundance of memory cells was produced during the primary response, where the antigen may be of lesser biological significance than in a secondary response. The authors discussed the fact that Pb may cause T cells to respond under conditions of low antigen concentration, which could waste valuable and limited resources by generating T memory cell clones when they are not needed (against unimportant antigens) or even increase the risk of autoimmune responses by altering the threshold requirements for stimulation. The putative mechanisms suggested for differential effects of Pb on Th cells are presented in Section 5.9.9.

5.9.5.3 Cytokine Production

At the time of the 1986 AQCD, immune cytokines were essentially absent from the information available for consideration. Only the antiviral interferons (α,β) had been examined among studies available for that report. Therefore, one of the most important effects of Pb on the immune system, i.e., Pb-induced cytokine production was not known at that time.

Most studies since 1986 have shown that Pb exposure at low to moderate levels causes a significant shift in the production of Th1 versus Th2 cytokines with the bias toward the latter. Hence, production of IFN is decreased and IL-12 is inadequate for effective host resistance. In contrast, production of IL-4, IL-6, and, frequently, interlukin-10 (IL-10) is elevated. Table 5-7 illustrates the studies reporting shifts in cytokine production induced by Pb. (Please note that TNF-α production is considered in the macrophage section, Section 5.9.6). These shifts in cytokine production are remarkably consistent, occur even at low levels of exposure, and are reported following both in vivo and in vitro exposure to Pb. Furthermore, the effects are persistent even when exposure to Pb was restricted to early development and cytokine assessment was performed in the subsequent juvenile or adult (Miller et al., 1998; Bunn et al., 2001c; Lee et al., 2001; Chen et al., 2004).

The only exceptions to Pb-induced biasing in favor of Th2 occur in the reports by Goebel et al. (2000) and Mishra et al. (2003). In the latter case, the authors attributed this difference (in humans) to the very high Pb levels considered in the study. In the prior case, Goebel et al. (2000) saw a local bias to Th1 in the intestinal tract of a specialized autoimmune diabetes-prone strain of mice (NOD) but not in normal mice. Initially, the Pb-induced cytokine skewing favored Th2 (after 1 day), but this shifted to Th1 with more prolonged Pb exposure (after 10 days). Loss of oral tolerance accompanied this long-term shift. These results suggest that in most cases, Pb-induced skewing would favor Th2. But with some genotypes or additional disease conditions, an imbalance may occur in the direction of a gut-associated Th1 environment, increasing risk for loss of oral tolerance and the potential for increased food allergies.

One ramification for the capacity of Pb to promote Th2 cells is the impact of elevated IL-4 on IgE. It seems clear that Pb-induced overproduction of IgE (seen in virtually all animal models examined as well as humans) is directly linked with the overproduction of IL-4. Excessive IL-4 and the resulting IgE production increase the risk for IgE-mediated atopy and asthma.

Table 5-7. Studies Reporting Lead-Induced Shifts in Th1 versus Th2 Cytokines

Species	Strain/Gender	Age	Cytokine Alterations	In vivo/ Ex vivo	Lead Dose/ Concentration	Duration of Exposure	References
Rat	F344 Females	Embryo-fetal	↑IL-4 ↓IFN-γ splenic lymphocytes	Yes	250 ppm in water to dams	2 weeks prior and 3rd week of gestation for dam	Chen et al. (2004)
Human	Males	Adults	↑ IFN-γ PHA stimulated peripheral blood lymphocytes	Yes	Not available	Not available	Mishra et al. (2003)
Chicken	Cornell K females	Embryonic	↓IFN-γ stimulated thymocytes	Yes	400 μg	Single injection E12	Lee and Dietert (2003)
Mouse	Balb/c	Neonatal/ Juvenile	↑IL-6 serum during infection	Yes	0.5 mM in water to dams and their pups	4 weeks (3 via dams)	Dyatlov and Lawrence (2002)
Rat	CD females	Fetal	↑IL-10	Yes	550ppm in water to dams	6 days via gestation of dam	Bunn et al. (2001c)
Chicken	Cornell K females	Embryonic	↓IFN-γ	Yes	50 μg	Single injection	Lee et al. (2001)
Mouse	Balb/c male	Adults	↑IL-6 serum during infection in certain groups	Yes	2 mM	8 weeks	Kim and Lawrence (2000)
Mouse	NOD Autoimmune strain adult	Adult	↓IFN-γ, no change long term ↓TGF-β intestinal levels	Yes	Oral 10 mM and ovalbumin antigen	10 days	Goebel et al. (2000)
Mouse	C57 Bl/6 females NOD autoimmune strain females	Adult	No effect on gut balance in normal mice ↓TGF-β in autoimmune mice	Yes	0.5 mg/kg injection and oral ovalbumin	6 injections over 2 weeks	Goebel et al. (1999)
Rat	F344 females	Embryo-fetal	↓IFN-γ ↑IL-10	Yes	250 ppm to dams	2 weeks before and 3rd week of gestation	Chen et al. (1999)

Table 5-7 (cont'd). Studies Reporting Lead-Induced Shifts in Th1 versus Th2 Cytokines

Species	Strain/Gender	Age	Cytokine Alterations	In vivo/ Ex vivo	Lead Dose/ Concentration	Duration of Exposure	References
Mouse	DO11.10 ova-tg, ova mice and RAG knockouts	Adult	↓IFN-γ	No	25 μM	3 days	Heo et al. (1998)
Rat	F344 females	Embryo-fetal	↓IFN-γ	Yes	500 ppm to dams	2 weeks before and 3rd week of gestation	Miller et al. (1998)
Mouse	Balb/c ByJ females	Adult	↓IFN-γ ↑IL-6	Yes	2 mM	3 weeks	Kishikawa et al. (1997)
Mouse	Balb/c and DO11.10 ova-tg mice	Adult	↓IFN-γ ↓IFN-γ/IL-4 ratio	Yes	50 μg each injection (s.c.) 3 per week	2 weeks	Heo et al. (1997)
Mouse	Balb/c ByJ female or male	Adult	↓IFN-γ ↑IL-4	Yes	50 μg each injection (s.c.) 3 per week	2 weeks	Heo et al. (1996)
Mouse	Balb/c ByJ female or male	Adult	↓IFN-γ ↑IL-4	No	10 μM – 50 μM	2 days	Heo et al. (1996)

Additionally, Kishikawa et al. (1997) demonstrated that administration of the potent Th1-promoting cytokine, IL-12, to Pb-exposed mice can restore the balance of Th1 (IFN-γ) versus Th2 cytokines (e.g., IL-6), reduce corticosterone levels, and enhance host resistance in *Listeria*-infected mice. This observation supports the critical role of Th1/Th2 balance in overall risk to host resistance against disease presented by Pb disruption of that balance

5.9.6 Macrophage Function

Macrophages represent a diverse population of cells that play critical roles in both host defense and tissue homeostasis. Macrophage subpopulations provide a front line of defense against bacteria, parasites, viruses, and tumors via the innate immune response. Additionally, they are important in tissue repair and remodeling as well as in the removal of senescent cells. Some forms of macrophages are efficient in the processing of antigens and the presentation of antigen fragments to T lymphocytes. Additionally, macrophages can regulate lymphoid activity through the secretion of a variety of cytokines and through the production of various immunomodulatory metabolites (e.g., NO, ROIs) and the products of the cyclooxygenase and lipoxygenase pathways.

Because macrophages can be found residing in most tissues, Pb-induced modulation of macrophage functional capacity has the potential to alter overall organ function. Macrophages originate in the bone marrow from pluripotent stem cells that give rise to both the monocyte-macrophage lineage as well as polymorphonuclear leukocyte populations. Bone marrow-derived macrophages mature under the influence of various cytokine growth factors to become the full array of mature cell subpopulations. Various investigators have examined Pb effects on the maturation of macrophages in vitro as well as on the functional capacity on fully mature cells both in vitro and in vivo. Blood monocytes represent a functional, yet not fully specialized, form of macrophage. As a result, the influence of environmental toxicants on monocytes may not be fully predictive of the effects of the same toxicants on splenic or alveolar macrophages, glial cells, or Kupffer cells.

Because macrophages give rise to several specialized populations, e.g., Kupffer cells in the liver, glial cells in the brain, and various skin macrophage populations, it is important to realize that different specialized macrophage populations are likely to have somewhat different sensitivities to Pb, as well as potentially different responses following exposure. Not too

surprisingly, blood monocytes may not always be an appropriate model to accurately predict the outcome of Pb-induced immunotoxicity for alveolar macrophages following inhalation exposure.

The 1986 Lead AQCD identified macrophages as a significant target for Pb-induced immunotoxicity. Research since the mid-1980s has served to underscore this point. The understanding of Pb-induced alterations in macrophage function has increased significantly since the 1996 AQCD report. The following sections describe the reported immunotoxic effects of Pb on macrophages. It should be noted that for a number of endpoints, such as Pb-induced alterations in the production of NO, ROIs and TNF-α, there is a general consensus among a majority of immunotoxicology studies and agreement with the effects described for the cardiovascular system (see Chapter 5.5).

5.9.6.1 Nitric Oxide (NO) Production

Nitric oxide is a short-lived metabolite produced in large quantities by macrophages during cellular activation. The enzyme responsible is an inducible form of nitric oxide synthase (iNOS), which, utilizing a bioptrin cofactor, converts the amino acid arginine into NO and citrulline. A competing alternative pathway utilizing arginine leads to the production of polyamines, which themselves are immunomodulatory for lymphocytes. Nitric oxide is critical in the defense against certain infectious agents, including various bacteria.

Among the most sensitive immunomodulatory effects of Pb exposure is the capacity to impair NO production by macrophages (Table AX5-9.6). Several research groups have shown that in vitro as well as in vivo exposure to Pb results in significantly reduced production of NO (Tian and Lawrence, 1995, 1996; Chen et al., 1997b; Lee et al., 2001; Pineda-Zavaleta et al., 2004 [also reviewed in Singh et al., 2003]). Similar results were obtained in human, mouse, rat and chicken. Depression of NO production capacity usually occurs shortly after exposure to lead. However, the long-term effects of Pb on NO production following very early life exposure are less clear (Miller et al., 1998; Chen et al., 1999; Bunn et al., 2001a).

Tian and Lawrence (1996) have hypothesized that because very low Pb concentrations (in vitro equivalents to 10 μg/dL) can impair NO production, impaired NO production may be responsible for reduced host resistance to *Listeria* seen among Pb-exposed rodents as well as for Pb-induced hypertension among humans (Pirkle et al., 1985). Indeed, impaired NO production

by macrophages seems to be one of the more sensitive endpoints for immediate Pb-induced immunotoxicity.

Other Functional Alterations

TNF-α Production

Early studies identified the fact that Pb exposure could predispose animals for a dramatically increased sensitivity to bacterially derived endotoxin (Trejo et al., 1972; Filkins and Buchanan, 1973; Schlick and Friedberg, 1981).

It is now known that the increased sensitivity to endotoxin is linked to the capacity of Pb to increase production of TNF-α among macrophages (Dentener et al., 1989; Zelikoff et al., 1993; Guo et al., 1996; Miller et al., 1998; Chen et al., 1999; Krocova et al., 2000; Flohé et al., 2002). Studies in mouse, rat, rabbit, and human provide a clear indication that one effect of Pb on macrophages is to boost production of the proinflammatory cytokine TNF-α. While most studies examined the immediate effects of Pb exposure on TNF-α production, studies by Miller et al. (1998) and Chen et al. (1999, (2004) showed that the effects of early gestational exposure to Pb on macrophages could persist well into later life, including adulthood. Also, Chen et al. (1999) showed that chelation of Pb with succimer in developing female rats in utero could eliminate the persistent effect of elevated TNF-α production in the adult offspring. Flohé et al. (2002) found evidence that Pb-induced elevation in TNF-α production is sensitive to both PKC signaling as well as to protein production. While the production of TNF-α can be elevated following exposure to Pb, the expression of the receptor for TNF-α (TNF-R) was also increased during the in vitro exposure of human blood monocytes to Pb-chloride (Guo et al., 1996). Therefore, the combined effect of elevated cytokine production by macrophages as well as increased receptor expression would be expected to contribute to problematic inflammatory responses.

Production of Other Proinflammatory Cytokines

Several studies have indicated that macrophage production of cytokines (or that levels of cytokines known to be produced primarily by macrophage populations) is altered after exposure to Pb. These vary somewhat, depending upon the exposure protocol and the source of macrophages examined. In addition to the previously discussed elevation of TNF-α by Pb, the

most significant and consistent Pb-induced effects seem to involve elevated production of the other major proinflammatory cytokines, interleukin-1β (IL-1β) and IL-6. Increased production of IL-6 following exposure to Pb has been reported by Dyatlov and Lawrence (2002), Flohé et al. (2002), Kim and Lawrence (2000), Krocova et al. (2000), Kishikawa and Lawrence (1998) and Kishikawa et al. (1997). Because IL-6 is a proinflammatory cytokine, its increased production following Pb exposure has the potential to influence many different tissues. Dyatlov et al. (1998a,b) provided evidence that Pb, IL-6 and LPS can combine to exert a significant impact on the permeability of the blood brain barrier as well as the properties of brain neurons and endothelial cells. Lead-induced elevation of IL-1β production has been reported by Dyatlov and Lawrence (2002). It is probable that enhanced co-production of IL-1β and IL-6 would increase the likelihood of local tissue inflammation.

Production of Reactive Oxygen Intermediates (ROIs)

Reactive oxygen intermediates (ROIs) are important metabolites in the capacity of macrophages and other inflammatory cells to kill invading bacteria and to attack cancer cells. However, increased overall production or inappropriate triggering of ROI release by macrophages can be a major contributor to tissue damage and the oxidation of cell surface lipids as well as DNA. The latter is one mechanism through which improperly regulated macrophages can actually increase the incidence of cancer. Results from many studies suggest that exposure of macrophages to Pb can increase the release of superoxide anion and/or hydrogen peroxide at least shortly after exposure. Key studies are summarized in Table AX5-9.6.

In a recent study on environmentally exposed children in Mexico, Pineada-Zavaleta et al. (2004) reported that production of superoxide anion by directly activated (interferon-gamma + LPS) monocytes was directly correlated with blood Pb level. This was in contrast with the effect of arsenic, which had a negative association. In other studies involving low levels of exposures, Zelikoff et al. (1993) demonstrated that rabbits exposed to Pb via inhalation had pulmonary macrophages that produced elevated levels of both H_2O_2 and superoxide anion upon stimulation in vitro. In an in vitro study, Shabani and Rabani (2000) reported that Pb nitrate exposure produced a dose dependent increase in superoxide anion by rat alveolar macrophages. Baykov et al. (1996) fed BALB/c mice dietary Pb and found that peritoneal macrophages had an increased spontaneous release of H_2O_2.

Other studies have reported no effects of Pb on superoxide anion production when a long recovery period was included following in vivo exposure (Miller et al., 1998) as well as negative effects of Pb on oxidative metabolism by certain macrophages or macrophage cell lines (Castranova et al., 1980; Hilbertz et al., 1986; Chen et al., 1997b). These somewhat different results suggest that the subpopulations of macrophages examined (e.g., alveolar versus splenic versus peritoneal) and the timeframe of assessment relative to exposure may be important factors in the effect of Pb on ROI production.

The biological importance of increased ROI production by Pb-exposed macrophages should not be underestimated. Fernandez-Cabezudo et al. (2003) showed that the potent antioxidant, vitamin E, could protect TO strain mice against some Pb-induced immunosuppressive alterations. Hence, macrophage-associated oxidative damage following exposure to Pb may be a mitigating factor in nonlymphoid organ Pb-induced pathologies.

Arachidonic Acid Content and Prostaglandin Production

Archidonic acid (AA) is a major surface component of many cells, including macrophages, and is the precursor of cyclooxygenase and lipoxygenase metabolites. As a result, the specific AA content of membranes and the capacity of macrophages to produce immunomodulatory metabolites from AA are important to overall health of the individual. One of the findings since 1986 concerning Pb-induced modulation of macrophage function is the impact of Pb on PGE_2 production. One study (Knowles and Donaldson, 1990) reported that diets supplemented with Pb at 500 ppm and fed to chicks produced an increase in the percentage of AA included in cell membranes. Such an increase would be expected to raise the risk of overall inflammation.

Several groups have reported that Pb exposure increases macrophage production of the immunosuppressive metabolite PGE_2. Lee and Battles (1994) reported that mouse macrophages exposed to Pb (10 μM) in vitro had elevated basal PGE_2 production, but under some stimulatory conditions, had decreased production of PGE_2. When Knowles and Donaldson (1997) fed Pb to turkey poults in the diet at a level of 100 ppm, macrophage production of prostaglandin F2 (PGF_2), PGE_2 and thromboxane production were all significantly elevated versus the control. Flohé et al. (2002) showed that exposure of mouse bone marrow-derived macrophages to Pb

chloride resulted in increased production of PGE_2 that correlated with increased mRNA production for the necessary enzyme, prostaglandin H synthase type-2.

Tissue Homeostasis

In an important observation reflecting the impact of Pb-induced immunotoxicity on nonlymphoid tissues, Pace et al. (2005) showed that neonatal exposure of mice to Pb acetate via drinking water (0.1 ppm for 6 weeks, both through maternal nursing and direct) produced a significant reduction in the testicular macrophage population. This correlated with increased estradiol levels in the testis and reduced male reproductive performance. The authors hypothesized that Pb-induced alteration among testicular macrophages is linked to an impaired tissue environment that likely includes increased oxidative stress, apoptotic somatic cells, and reduced fertility of males.

Colony Formation and Population Distribution

The ability of bone marrow-derived macrophages (BMDM) to form colonies in response to certain growth factors (e.g., colony stimulating factor-1 [CSF-1]) is a property related to the growth and differentiation of subsequent macrophage populations. Kowolenko et al. (1991) found that exposure to CBA/J female mice to Pb acetate (0.4 mM in drinking water for 2 weeks) reduced colony formation of macrophages in response to CSF-1. Infection of the mice with *Listeria* only exacerbated this effect of Pb. The same authors (Kowolenko et al., 1989) had previously demonstrated that when BMDM were cultured in vitro with Pb chloride (0.1 μM), colony formation was significantly impaired. These combined results suggest that exposure to Pb can impair the generation of macrophage populations as well as modulate the functional spectrum of fully matured macrophages. Bunn et al. (2001a) reported that gestational exposure of CD rats to 50 ppm Pb acetate via the drinking water of the dams resulted in female adult offspring with a significantly decreased percentage (58% reduced) of circulating monocytes. A 100-ppm dose of Pb acetate produced a significant reduction (74% reduced) in the absolute numbers of monocytes as well. The blood Pb level at birth associated with the decreased percentage of macrophages in the adult offspring was 8.2 μg/dL. In general agreement, Lee et al. (2002) reported a significant decrease in the absolute numbers of circulating monocytes and polymorphonuclear leukocytes (PMNs) in juvenile female chickens exposed in ovo on

embryonic day (E) 12 to 200 µg Pb acetate. The corresponding blood Pb level at hatching was 11.0 µg/dL. However, in this case, the Pb-induced reduction in monocytes and PMNs was only seen in concert with an airway viral infection (viral stressor) and not in the resting uninfected animal.

Antigen Presentation and Lymphoid Stimulation

Exposure to Pb influences the interaction between macrophages and T lymphocytes, and as a result, the capacity of macrophages to support T lymphocyte proliferation and activation can be altered as well. Kowolenko et al. (1988) found that mouse macrophages exposed to Pb (both in vivo and in vitro) can induce an increased proliferative response of T lymphocytes in co-culture but that antigen-specific stimulation of primed T cells is significantly reduced. Lead-suppressed antigen presentation capabilities of mouse macrophages were also reported by both Smith and Lawrence (1988) and Blakley and Archer (1981).

Chemotaxis

Chemotactic activity of macrophages is an important function required for the directed migration of macrophages to sites of infection and tumor growth. However, it is a functional capacity that has not been systematically examined within the lead-immune literature. Using female Moen-Chase guinea pigs, Kiremidjian-Schumacher et al. (1981) showed that Pb chloride exposure of peritoneal macrophages in vitro (10-6 µM) inhibited the electrophoretic mobility of the cells.

Phagocytosis and Clearance of Particles

Phagocytosis of targets and removal/clearance of dead cells and particles are major functions of macrophages. However, phagocytosis can involve a variety of different cell surface receptors on macrophages, depending upon both the nature of the target encountered and the subpopulation of macrophages examined. In general, phagocytic capacity of macrophages seems to be relatively insensitive to Pb-induced immunomodulation compared with the effects on NO and TNF-α production.

However, differences in outcome in phagocytosis evaluations are likely to be based on the differences in the source of macrophages used and their relative activation state at the time of

assessment. A few studies have described significant effects on phagocytosis, but these have usually relied upon phagocytosis mediated through the Fc receptor on macrophages. Because cell adherence to surfaces may be influenced negatively by Pb (Sengupta and Bishali, 2002), impairment of phagocytosis may also involve some lack in efficiency with macrophage anchoring to substrates. De Guise et al. (2000) reported no effect on bovine macrophage phagocytosis of latex beads by Pb at in vitro treatment concentration of 10^{-4} M. This was in contrast with suppressive effects of both cadmium and mercury. Using Sephadex-elicited peritoneal macrophages derived from young turkeys fed 100 ppm Pb in the diet, Knowles and Donaldson (1997) found a 50% reduction in the percentage of phagocytic macrophages using SRBC targets. The activity per phagocytic macrophage was also reduced.

Kowolenko et al. (1988) studied the effect of Pb acetate at 10 mM in the drinking water of CBA/J mice. They reported no effect on phagocytosis of *Listeria monocytogenes* targets, yet they found an overall decreased resistance to *Listeria.* When the same investigators exposed peritoneal and splenic macrophages to Pb in vitro (100 µM), they also found no significant effect of Pb on phagocytic activity. Zhou et al. (1985) reported that New Zealand white rabbit-derived alveolar macrophages exposed to Pb in vitro at 10^{-5} M concentration were significantly impaired in the phagocytosis of opsonized chicken erythrocytes (Fc receptor-mediated phagocytosis). Trejo et al. (1972) reported that a single i.v. injection of Pb (5 mg/rat) into male Sprague Dawley (SD) strain rats produced an inhibition in the phagocytic capacity of Kupffer cells.

Several studies have reported a decreased clearance capacity of the reticuloendothelial system following in vivo exposure to Pb. Filkins and Buchanan (1973) found that injection of 5 mg of Pb acetate i.v. into male Holtzman strain rats produced reduced carbon clearance. Similarly, Trejo et al. (1972) reported that a single i.v. injection of Pb (2.5 mg) into male SD strain rats significantly reduced clearance of colloidal carbon.

In contrast, Schlick and Friedberg (1981) found that 20 µg/kg Pb acetate in a single i.p. injection of NMRI strain mice significantly increased the clearance of India ink. Ironically, oral administration of Pb for 10, but not 30, days of 10 µg/kg resulted in an increase in clearance activity. Difference in route of Pb administration may be a factor in the different results obtained.

Induction of Heat Shock Proteins

One study (Miller and Qureshi, 1992), using a macrophage cell line, reported that exposure of macrophages (MQ-NCSU) in culture to Pb acetate (1000 μM) induced the same set of four heat shock proteins as when the macrophages were subjected to thermal stress. This result fits the hypothesis that Pb produces a profound immunomodulatory effect in macrophages that has similarities with the exposure of macrophages to certain pathogens.

Apoptosis

Significant differences exist in the literature concerning the potential role of Pb in the apoptosis of macrophages. The difference may be based on the exposure methodologies (in vivo versus in vitro) as well as the source of macrophages utilized. De la Fuente et al. (2002) found that human monocytes exposed to Pb in vitro at high concentrations did not undergo apoptosis. This was in direct contrast with the apoptosis-promoting effects of cadmium in the same assessment protocol. In contrast, Shabani and Rabibani (2000) exposed rat alveolar macrophage to Pb nitrate in vitro and found that 60 μM concentration produced a significant increase (2x) in DNA fragmentation after 3 to 24 h in culture.

5.9.7 Granulocytes and Natural Killer (NK) Cells

Other cell types important in innate immunity, as well as in immunoregulation, are the lymphoid population of natural killer cells and granulocytes, including PMNs (i.e., neutrophils). Neither population appears to be a major target for Pb-induced immunotoxicity, although both may be influenced indirectly via immune cell-cell interactions as well as by changes in cytokine production. Among the two, neutrophils may be the more sensitive cell type based on assays conducted to date. For neutrophils, several groups have reported alteration in chemotactic activity following exposure to Pb. Queiroz et al. (1993) found impaired migration ability of neutrophils from battery workers occupationally exposed to Pb. Likewise, Valentino et al. (1991) had a similar observation among male occupationally exposed workers. Lead exposure of young SD strain rats can increase the population of neutrophils (Villagra et al., 1997), although, as the authors indicated, this does not necessarily afford enhanced host protection against disease. Baginski and Grube (1991) reported that human neutrophils exposed to Pb had increased killing capacity, probably via increased release of ROIs despite having reduced

phagocytic capacity. This would fit the same general profile as the Pb effects on macrophages. Therefore, neutrophils may contribute to Pb-induced tissue inflammation and damage via increased ROI release. Yet, their effectiveness in protection against disease challenge may be no greater following exposure to Pb, because some impairment in chemotaxis and phagocytosis has been reported as well.

Yucesoy et al. (1997) reported that either Pb exposure or simultaneous exposure to Pb and cadmium in human workers did not impair NK cytotoxicity activity. This finding was supported by studies using in vivo exposure to Pb in rats (Kimber et al., 1986) and mice (Neilan et al., 1983). Therefore, it would appear that NK cells are not a prime target associated with Pb-induced immunotoxicity, although more subtle effects may certainly exist within the cell type.

Eosinophils represent an important granulocytic cell type in type 2 associated inflammatory and allergic reactions. However, few studies have examined Pb exposure and eosinophil activity. Villagra et al. (1997) reported that exposure of female juvenile SD rats to Pb [four alternate-day s.c. injections of 172 mg/g body wt Pb acetate] increased the degranulation of eosinophils (in animals given estrogen 1 day later). Such a response would be expected to contribute to increased inflammation.

5.9.8 Hypersensitivity and Autoimmunity

At the time of preparation of the 1986 AQCD, little was known about the potential for Pb to influence the risk of allergic and autoimmune diseases. However, since the early 1990s, a significant number of studies have all pointed toward the fact that Pb causes a profound dysregulation of the immune system. It skews the balance of responses in directions that reduce certain host defenses against infectious diseases while enhancing the risk of allergic and autoimmune disease. Lead exposure at low to moderate levels appears to alter T lymphocyte responses in such a way as to increase the risk of atopy, asthma, and some forms of autoimmunity. Increased IgE production following exposure to Pb is among the most frequently reported immune alterations. Elevated IgE levels would be an associated risk factor for atopy and allergic disease. Several investigators have discussed the fact that Pb is a likely risk factor associated with the increased incidence of childhood allergic asthma (Miller et al., 1998; Heo et al., 1998; Snyder et al., 2000; McCabe et al., 2001; Dietert et al., 2004; Trasande and Thurston, 2005) as well as later life allergic disease (Heo et al., 2004; Carey et al., 2006).

Joseph et al. (2005) observed no association for childhood blood Pb concentration and risk of asthma among an African-American population. However, results on other populations from this study, including those involving Caucasian children with blood Pb levels above 5 µg/dL, led the authors to call for further studies into the possible linkage of early life Pb exposure and risk of asthma (Joseph et al., 2005).

As described by McCabe et al. (1991) and discussed by Dietert et al. (2004), Pb-induced immunotoxicity is novel in that profound cellular toxicity is not evident following exposure at low to moderate exposure concentrations. In fact, antibody responses overall are usually unaffected or may be increased depending upon the class/isotype measured. However, the functional responses mounted following Pb exposure do not reflect the normal immune balance that would otherwise occur. This dysregulation can alter the risk of certain autoimmune diseases based on several observations. Holladay (1999) has considered the importance of the timing of exposure and the fact that early life exposure may establish the immune profile that then contributes to later disease including autoimmunity.

Hudson et al. (2003) reported that Pb exposure can exacerbate systemic lupus erythmatosus (SLE) in lupus-prone strains of mice. In contrast with the effect of mercury, these authors found that for lupus, Pb exposure would not induce this autoimmune condition in genetically resistant mice but would increase severity of the disease in genetically prone animals. The authors noted some gender effects within certain strains (e.g., NZM88). Using early in ovo exposure to Pb (10 µg/egg), Bunn et al. (2000) found that Pb acetate-exposed male chicks could be induced to produce autoantibodies against thyroglobulin, which were not present in acetate-exposed controls. No Pb-induced alteration was observed in females that were predisposed to mount anti-thyroglobulin responses. The gender effect is intriguing in that autoimmune thyroiditis in genetically predisposed strains is always more severe in females than in males.

Two lines of evidence suggest that the capacity of Pb to influence the risk of autoimmunity is not always associated with simply a strict shift from Th1 to Th2 responses. Hudson et al. (2003) discussed the fact that lupus is not purely a Th2-mediated disease, but rather seems to occur under conditions associated with skewing in either direction. McCabe et al. (2001) found that Pb can increase the stimulation of alloantigen reactive T cells (where macrophage processing of antigen is required) but not enhancement of T cell clonotypic responses against either mitogens or superantigens (where processing is not required).

This suggests that the role of Pb in influencing risk of autoimmune disease goes beyond a simple consideration of Th1/Th2 balance. In fact, Goebel et al. (2000), studying mucosal immunity, reported that administration of Pb-chloride to NOD strain mice produced a gut cytokine microenvironment that was skewed toward Th2 over the short run, but later was shifted toward Th1 with increased production of IFN-γ. This shift to Th1 was accompanied by a loss of tolerance and capacity to mount an immune response against a diet-associated protein (chicken ovalbumin). The authors proposed that reduction of the capacity for oral tolerance would predispose an individual toward autoimmune disease. The findings of Carey et al. (2006) had similar implications. These authors reported that exposure of mice to Pb chloride increased activation of neo-antigen-specific T cells, thereby increasing the risk of autoimmunity.

Finally, Waterman et al. (1994) and El-Fawal et al. (1999) have described the production of autoantibodies against neural proteins in both battery workers and rats exposed to low levels of Pb via drinking water. These authors have suggested that exposure to Pb may precipitate the autoimmunity by altering antigen immunogenicity and/or the capacity of the immune system to respond to certain antigens. This, in turn, may contribute to the eventual Pb-associated neurological disease.

5.9.9 Mechanism of Lead-Based Immunomodulation

In the 1986 AQCD, there was little direct information available about the immune system regarding the molecular mechanism(s) of Pb-induced immunotoxicity. Binding to thiol groups and altering cell surface receptors were indicated as possible factors in altered immune function. Since that time, some additional information has been generated through a variety of studies on human and animal immune cells. However, a clear or simple explanation remains to be determined. Table 5-8 lists studies on the immune system that have contributed to a better understanding of potential mechanisms or have forwarded potential hypotheses with some supporting data.

At the level of cell-cell interactions, it seems clear that Pb alters metabolism and cytokine production by macrophages and antigen presenting cells. It also reduces their capacity to respond to growth factors such as CSF-1 (Kowolenko et al., 1989). Pace et al. (2005) discussed the hypothesis that reduced populations of functionally altered macrophages (because of Pb-induced unresponsiveness to CSF-1and over production of ROIs) in tissues can produce

Table 5-8. Suggested Mechanisms of Lead-Induced Immunotoxicity

Species	Strain/Gender	Suggested Endpoints	Associated Functional Alteration	Lowest Effective Dose	Duration	References
Mouse	Balb/c	CSF-1 Responsiveness of Macrophages	↓Testicular macrophages ↓Fertility	0.1 ppm	6 weeks	Pace et al. (2005)
Mouse	TO strain males	Vitamin E protection against lead-induced splenomegaly	↑Putative ROI associated splenomegaly	1 mg/kg	2 weeks	Fernandez-Cabezudo et al. (2003)
Chicken	Cornell K Strain	Thymulin partial reversal of Th skewing	↓Lead-induced DTH suppression	400 µg	Single in ovo injection	Lee and Dietert, (2003)
Mouse	Balb/c females C57 Bl/6 females	Lead disruption of antigen processing and presentation signals	↑Alloreactive CD4+ ^{high} cells ↑Risk of autoimmunity	0.5 µM in vitro	4 days	McCabe et al. (2001)
Mouse	C 57Bl/6	PKC activation	↑TNF-α, ↑IL-6 ↑PGE_2	20µM in vitro	4.5 hrs	Flohé et al. (2002)
Rat	PC-12 cells	NF-κB activation AP-1 induction C-Jun kinase induction	↑ROI	1 µM in vitro	5-120 min	Ramesh et al. (1999)
Mouse	DO11.10 ova-mice	Adenylcyclase activation with elevated cAMP levels	↑Th skewing	2.5 µM in vitro	15 mins-6 hrs	Heo et al. (1998)
Mouse	DO11.10 ova-tg mice	Vβ gene usage	↑Risk of autoimmunity	50 µg 2x/week s.c.	8 weeks	Heo et al. (1997)
Human	-	NF-κB activation in CD4+ cells	↑Risk of autoimmunity and hypersensitivity	1 µM	30 min	Pyatt et al. (1996)
Mouse	CBA/J females	↑Immunogenicity of neural proteins	↑Autoimmune mediated neurological damage	Lead-altered proteins used as antigens	3 injections of lead-modified neural proteins	Waterman, et al. (1994)
Mouse	Swiss Females	↑TNF-α production	↑Sensitivity to endotoxin	5 mg	Single i.p. injection	Dentener et al. (1989)

nonimmune problems. The model they used is the homeostatic presence of testicular macrophages and the likelihood that Pb-induced macrophage immunotoxicity contributes directly to Pb-associated reduction in male fertility.

Also, Pb is known to selectively alter cell signaling to CD4+ T cell subpopulations, promoting proliferation in some but not others. The outcome is enhanced tissue inflammation, reduced CMI, and increased production of atopy-inducing antibodies. Risk of autoimmune reactions is increased in some models of Pb-induced immunotoxicity. For example, Heo et al. (1997) reported that Pb-exposed murine T lymphocytes are biased in expression of Vβ genes. This is potentially problematic as this phenotype is common among a variety of human and animal model autoimmune conditions. A variety of exogenous factors has been reported to partially ameliorate Pb immunotoxic effects. Lead chelation in Pb-exposed dams corrected some Pb-induced immunotoxic problems in the rat female offspring, but it left the animals with some DMSA-induced immune alterations (Chen et al., 1999). Other exogenously administered factors that have been reported to partially restore Pb-suppressed immune function are vitamin E (Fernandez-Carbezudo et al., 2003) and thymulin (Lee and Dietert, 2003).

At the subcellular level, the bases for immunotoxic changes remain speculative. McCabe et al. (2001) suggested that altered antigen processing and subsequent cell signaling to T cells may be an explanation for the capacity of Pb to selectively increase CD4+ (high density) cells. Certainly, Pb appears to alter signal transduction. It appears to elevate expression of the nuclear transcription factor NF-κB (Pyatt et al., 1996; Ramesh et al., 1999) as well as to increase expression of AP-1 and cJun (Ramesh et al., 1999). Flohé et al. (2002) found evidence that Pb can elevate the activation of PKC. The authors speculated that this might be involved in Pb-induced increases in TNF-α production. Additionally, Heo et al. (1998) reported that Pb increases adenyl cyclase activity among T lymphocytes, generating elevated cAMP levels. The authors hypothesized that this effect, in conjunction with differences in cell signaling pathways for promoting Th1 versus Th2 cells, may be involved in the capacity of Pb to skew Th0 helper cells toward Th2.

5.9.10 Age-Based Differences in Sensitivity

With the literature available at the time of the 1986 AQCD, it was virtually impossible to evaluate age-based differences in susceptibility to Pb-induced immunotoxicity. However, in

recent years, this has become a major topic of study for many toxicants, including Pb (Dietert and Piependrink, 2006). Several studies have added to the available data assessing the developmental immunotoxicity of Pb (reviewed in Barnett [1996], Dietert et al. [2000, 2004], Lee and Dietert [2003]). Several patterns have emerged from exposure data using animals of different ages.

First, it seems clear that blood Pb levels at or near birth of below 10 µg/dL can be associated with juvenile and/or adult immunotoxicity. Several studies reported effects at blood Pb levels in the range of 5 to 8 µg/dL. These low levels would seem to place the immune system on par with the nervous system in terms of potential sensitivity to Pb. Table 5-9 shows examples of studies in which low blood Pb levels were linked with immunotoxicity.

Table 5-9. Immunomodulation Associated with Low Blood Lead Levels in Animals

Species	Blood lead (µg/dL)	Age at Measurement	Immune Parameter(s)	Age at Assessment	Reference
Mouse	~5.0	1 week	↑IgE, ↓ Splenic T Cell Populations	2 weeks	Snyder et al. (2000)
Rat	8.2	1 day	↓monocytes	13 weeks	Bunn et al. (2001a)
Rat	6.75	4 weeks	↓DTH, ↓IFN-γ, ↑IL-4	13 weeks	Chen et al. (2004)
Rat	8.0	4 weeks	↑TNF-α ↑Rel. Spleen weight	13 weeks	Lee et al. (2002)
Chicken	8.2	1 day	↓circulating lymphocytes post infection	5 weeks	Lee et al. (2002)
Chicken	11.0	1 day	↓DTH and ↓TLC, monocytes, PMNs post infection	5 weeks	Lee et al. (2001)
Chicken	7.0	1 day	↑autoantibody production	10 weeks	Bunn et al. (2000)

A second finding is that the immunotoxic effects induced by Pb are persistent long after blood levels and potential body burdens of Pb are significantly reduced. Miller et al. (1998), Chen et al. (1999), Snyder et al. (2000), and Lee et al. (2001) all emphasize this latter point.

In fact, in most of these studies immunotoxic alterations were present when Pb levels in exposed animals were not distinguishable from control levels. This should provide a cautionary note regarding studies in humans. Data from adult exposures provide little insight into the potential persistence following adult exposure to Pb. However, rather than the developing immune system being more regenerative postexposure and able to withstand immunotoxic insult, it appears that the non-dispersed developing immune system is a particularly susceptible target to many immunotoxicants (Dietert et al., 2002).

A third, and somewhat surprising, finding concerning early exposure to Pb is that qualitative differences in the spectrum of immune alterations can exist, depending upon the developmental window of exposure. Figure 5-17 illustrates this point. Early embryonic exposure of rats and chickens to Pb failed to alter juvenile DTH responses, despite significant effects on macrophage function. However, exposure to Pb after the mid-embryonic point of embryonic development readily suppressed subsequent DTH. As shown in Figure 5-17, the development window in which sensitivity to DTH suppression emerges is quite similar in the two species. This observation suggests that both quantitative (LOAELs) and qualitative (range of immune alterations) differences in sensitivity to Pb can exist across different age groups.

Additionally, some studies in animals have noted gender differences in the effects of Pb following exposure (Bunn et al., 2000, 2001a,b, c; Hudson et al., 2003). Gender differences have also extended to results in humans as per Pb-induced immune and inflammatory alterations (Karmaus et al., 2005; Fortoul et al., 2005). It seems feasible that, even in the embryo, hormonal differences among females and males may impact some outcomes of low-level Pb exposure.

Table 5-10 shows comparisons of the lowest reported blood Pb levels at different ages associated with the same immunotoxic endpoint. From these limited comparisons, it would appear that different ages of rodents (e.g., embryonic versus adult) differ in dose sensitivity for Pb-induced immunotoxicity somewhere in the range of 3 to 12-fold. Clearly, additional direct comparisons would help to refine this estimate.

A fourth observation from the early exposure studies is that exposure to even very low levels of Pb can predispose the immune system for unanticipated postnatal responses when the system is stressed. This general phenomenon is called latency. Lee et al. (2002) provided an example of this following the single in ovo exposure of embryonic day 5 chick embryos to low levels of Pb (10 μg; blood lead level 1 day post hatch of 8.2 μg/dL). The leukocyte profiles of

Table 5-10. Comparisons of Age-Based Sensitivity to Lead-Induced Immunotoxicity

Species	Altered Endpoint	Embryo – fetal*	Neonatal*	Adult*	References
Mouse	↑IgE	~5μg/dL	12 μg/dL	38 μg/dL	Snyder et al. (2000) Heo et al. (1996)
Rat	↓DTH (persistent effect assessed 13 weeks post-exposure)	34 μg/dL	—	>112 μg/dL (measured at birth for persistent effect)	Miller et al. (1998) Bunn et al. (2001b)
Mouse	↓DTH	—	29 μg/dL	87 μg/dL	Faith et al. (1979) McCabe et al. (1999)
Rat	↑TNF - α (persistent effect assessed 13 weeks post-exposure)	8 μg/dL	—	>112 μg/dL (measured at birth for persistent effect)	Miller et al. (1998) Chen et al. (2004)

*Lowest blood lead concentration reported with effect.

the animals appeared to be completely normal. However, when these animals were exposed to a respiratory virus, their pattern of leukocyte mobilization was completely aberrant from controls. Therefore, some immunotoxic alterations following early exposure to low levels of Pb may only be evident during periods of postnatal stress.

Several studies have reported the positive association of blood Pb levels in children with elevated serum IgE (Karmaus et al., 2005; Sun et al., 2003; Lutz et al., 1999). These observations are supported by the animal data in rats and mice (Miller et al., 1998; Snyder et al., 2000) and suggest that Pb-induced risk of atopy and asthma may be a particular health issue.

Trasande et al. (2005) recently discussed the fact that, despite progress in reducing the deposition of Pb in the environment, Pb continues to be a concern relative to asthma and children's health.

5.9.11 Summary

The immune system appears to be one of the more sensitive systems to the toxic effects of Pb. The 1986 AQCD provided an excellent summary of the studies that had been conducted prior to that date. But knowledge of fundamental immunology has progressed greatly during the

past 20 years. Not surprisingly, the large number of studies conducted since the mid-1980s have provided a much clearer understanding of the immune-associated problems that can arise from problematic exposure to Pb. Studies across humans and a variety of animal models are in general agreement concerning both the nature of the immunotoxicity induced by Pb as well as the exposure conditions that are required to produce immunomodulation. Figure 5-18 summarizes the basic immunotoxic changes induced by Pb that result in Th skewing, impaired macrophage function, and increased risk of inflammation-associated tissue damage.

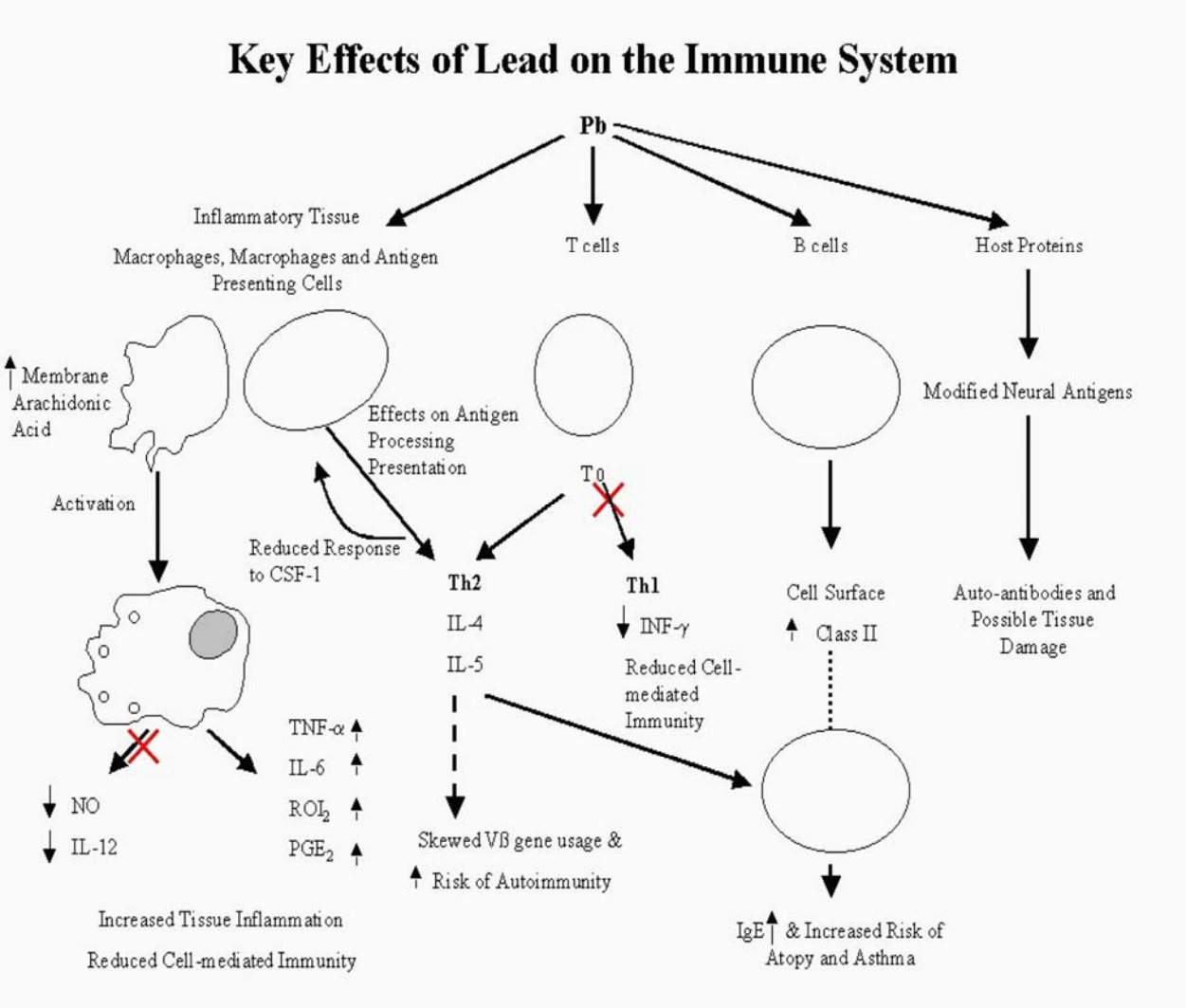

Figure 5-18. This figure shows the fundamental alterations to the immune system and to immunological response and recognition induced by exposure to lead. The functional shifts are disproportionate compared to the relatively modest changes among leukocytes with low to moderate exposure to lead.

- Lead is unlike many immunotoxicants in that, at low to moderate levels of exposure, it does not produce overt cellular cytotoxicity or lymphoid organ pathology. However, it can induce profound functional alterations that influence risk of disease. Lead preferentially targets macrophages and T lymphocytes, although effects have been reported in B cells and neutrophils as well.

- There are three major hallmarks of Pb-induced immunotoxicity. First, Pb can dramatically suppress the Th1-dependent DTH response, as well as production of associated Th1 cytokines. Second, Pb can dramatically elevate production of IgE while increasing production of Th2 cytokines, such as IL-4. Third, and perhaps most sensitive, is the modulation of macrophages by Pb into a hyperinflammatory phenotype. After exposure to Pb, macrophages significantly increase production of the proinflammatory cytokines TNF-α and IL-6 (and in some studies IL-1). Many studies also reported elevated release of ROIs and prostaglandins. Ironically, production of one of the most important host defense factors, NO, is consistently and severely suppressed by Pb exposure. This package of Pb-induced changes among macrophages makes them more prone to promote tissue destruction but actually less capable of killing bacteria or possibly presenting antigens to T lymphocytes. The Pb-induced shift in phenotype explains the capacity of inhaled Pb to promote bronchial inflammation while bacterial resistance is severely depressed.

- Lead-induced skewing of Th activity (biasing responses toward Th2) across a population argues for expectation of a greater risk of atopy, asthma, and some forms of autoimmunity. Concomitantly, resistance to some infectious diseases could be reduced. This predicted change of risk might help explain some recent trends in the incidence of diseases, such as the epidemic rise in allergy and some forms of asthma in the United States at current blood Pb levels well below 10 µg/dL.

- Sensitivity of the immune system to Pb appears to differ across life stages. Studies in rats and mice suggest that the gestation period is the most sensitive life stage, followed by the early neonatal stage. But even during embryonic, fetal, and early neonatal development, critical windows of vulnerability are likely to exist. Compared to adults, the increased dose sensitivity of the embryo-fetus would appear to fall in the range of 3-10x depending upon the immune endpoint considered. Some studies have found evidence for gender differences in the impact of Pb on the immune system, particularly with early life exposures. Potential gender differences in immunotoxic outcome may be important in the evaluation of those populations at greatest risk.

- Recent studies have suggested that exposure of embryos to Pb producing neonatal blood Pb levels below 10 µg/dL can also produce later-life immunotoxicity (see Table 5-9). Furthermore, immunotoxicity persists long after any evidence of prior embryonic Pb exposure. This latter observation from several laboratories may have implications for the design of human epidemiological studies.

5.10 EFFECTS OF LEAD ON OTHER ORGAN SYSTEMS

In the 1986 Lead AQCD, discussion of other organ systems included the cardiovascular, hepatic, gastrointestinal (GI), and endocrine systems. Due to our increased understanding on the effects of Pb on cardiovascular and renal systems and their contribution to potential health effects of Pb, separate sections (5.5, 5.7) were dedicated earlier in this chapter to detailed discussions on these aspects. Similarly, with our increased understanding on the effects of Pb on endocrine functions and its inherent role with respect to neurotoxicological, reproductive, and developmental effects, literature reviewed for Pb endocrine effects was discussed in the respective sections. This section focuses on the discussion of Pb effects on the hepatic and GI systems.

5.10.1 Effects of Lead on the Hepatic System

The liver is a highly active metabolic tissue. Apart from its roles in fatty acid metabolism and limited heme synthesis function, the liver also has a major role in guarding other systems from the toxic effects of xenobiotic compounds using a huge complement of detoxification machinery referred to as phase I and phase II enzyme systems. Limited studies on experimental animals reported in the 1986 AQCD indicated that Pb induced effects in the hepatic system. Laboratory animals, especially rats, exposed to Pb nitrate have exhibited increased liver cell proliferation, DNA synthesis, cholesterol synthesis, and glucose -6-phosphate dehydrogenase (G6PD) activity indicative of Pb-induced hyperplasia. Further, the literature reviewed in the 1986 AQCD reported alterations in the levels of drug metabolizing enzymes in experimental animals given large doses of Pb. The evidence for such effects in humans was less consistent. The 1986 document also concluded that the effects on the liver occurred only at high exposure levels. The majority of studies on the effects of Pb on the hepatic system in experimental animals that are reviewed in this document report functional and biochemical changes in the liver, clearly pointing to metabolic perturbations in liver. For ease in understanding and integration of these functional changes, the discussion is divided into the following four subsections: hepatic drug metabolism, lipid and glycogen metabolism and lipid peroxidation, and heme synthesis.

5.10.1.1 Hepatic Drug Metabolism

Approximately 75% of the hepatic blood comes directly from the gastrointestinal viscera, with the majority of drugs or xenobiotics absorbed coming directly to the liver in concentrated form. The liver is equipped with a huge complement of drug metabolizing enzymes that detoxify many of the xenobiotics but also activate the toxicity of others. Oxidation and conjugation of xenobiotics have historically been referred to as phase I and phase II reactions. The phase I enzymes include cytochrome P450 (CYP450) heme-containing monoxygenases, flavin-containing monoxygenases, and epoxide hydrolases. The phase II enzymes include glutathione (GSH) S-transferases (GST), UDP-glucuronyl transferases (UGT), N-acetyltransferases (NAT), and sulfotransferases (SULT). Xenobiotic metabolism by these two complements of enzyme systems are essential for catabolizing and eliminating drugs; however, this process can also produce activated toxicants and carcinogens. A limited number of these CYP450s are involved in the biosynthetic pathways of steroid and bile acid production. It has been increasingly recognized that, under certain circumstances, CYP P450s can produce ROS that result in oxidative stress and cell death.

Liver is an active tissue. In addition to xenobiotic metabolism, it also participates in gluconeogenesis, fatty acid metabolism, and cholesterol biosynthesis. Research concerning the effects of Pb on the hepatic system in the past 15 years has provided some preliminary indications of Pb-induced alterations in many of the hepatic functions described above.
The following discussion presents, as much as possible, the effects of Pb on individual enzymes, but due to the multifarious interactions of many of these metabolic enzymes, there may be places such separation was not possible.

Phase I Enzyme

Earlier studies on the toxic effects of Pb on hepatic drug metabolizing enzymes demonstrated that acute exposure to Pb acetate decreased rat hepatic CYP450s with increased levels of urinary δ-aminolevulinic acid (ALA). Co-treatment with phenobarbitol, a CYP450 inducer, was shown to reverse the decrease CYP450 levels, suggesting a Pb acetate-mediated inhibition of heme synthetic enzymes. Decreased activities of estradiol-17 beta enzyme observed in rat liver treated with triethyl Pb chloride (Odenbro and Arhenius, 1984) suggest that both Pb and organo-Pb compounds are capable of inhibiting CYP450 activities. Roomi et al.

(1986) also observed decreased levels of hepatic microsomal CYP450s and decreased aminopyrene-N-demethylase activity on exposure to a single dose of Pb nitrate (5–10 mmol/kg body wt). This decrease in phase I enzymes was followed by increased levels of phase II components such as GSH, GST, and DT diaphorase, suggesting that Pb nitrate and other Pb compounds can induce biochemical properties characteristic of hepatocyte nodules. Subchronic (2–3 months) exposure to Pb acetate (5-50 mg/kg body wt) had been found to induce CYP450s and cytochrome b5 in rat liver and kidney (Nehru and Kaushal, 1992). As described earlier, multiple isoforms of CYP450s exist in the liver.

To identify the inhibitory effect of acute Pb exposure on specific isoform(s), Degawa et al. (1994) exposed male F344 rats to Pb nitrate (20,100 µmol/kg body wt) and evaluated liver CYP450s 24 h postexposure. Lead nitrate exposure preferentially inhibited cytochrome P4501A2 enzyme activity in liver microsomal preparations as assayed for mutagenic conversion of substrates 2-amino-6-methyl-dipyridol [1,2-a; 3',2-d] imidazole and 3-amino-1-methyl-5H-pyridol [4,3,-b] indole. Lead nitrate exposure also inhibited the induction of cytochrome P4501A2 by the inducers 3-methylcholanthrene and 2-methoxy-4-aminoazobenzene at both the protein and mRNA levels. The authors further concluded that the specific inhibition of P4501A2 by Pb nitrate observed may have been due to inhibition of heme synthesis, as Pb nitrate was not found to inhibit P4501A2 activity in vitro. Additional studies carried out by the same group using various metal ions (e.g., Pb, Ni, Co, and Cd) found that the specific inhibition of P4501A2 was unique to Pb nitrate (Degawa et al., 1994, 1995). Degawa et al. (1996) also investigated the effect of Pb nitrate-mediated inhibition of CYP1A gene activity in rat liver by specific inducers and reported that Pb nitrate inhibited the induction of CYP1A mRNA by aromatic amines, but not by aryl hydrocarbons, suggesting the role of other cellular factors in the transcriptional activation of CYP1A genes. Lead nitrate has been reported to induce the production of TNF-α in rat liver (Shinozuka et al., 1994), a cytokine implicated in the suppression of constitutive expression of CYP1A2 mRNA in rat hepatocytes. Based on these findings, Degawa et al. (1996) concluded that the inhibition of constitutive and aromatic amine-induced expression of CYP1A2 in rat liver caused by Pb nitrate may occur at least in part by TNF-α-associated mechanisms. Lead nitrate (0.33 mg/kg body wt) pretreatment-mediated protection conferred against carbon tetrachloride (0.3 mL/kg)-induced hepatotoxicity as reported by Calabrese et al. (1995) may be due to the inhibition of CYP450 activities in liver by Pb.

Jover et al. (1996) investigated the effect of heme deficiency on Pb-induced hepatic P450 function and transcription. These authors concluded that the decrease in hepatic P450 resulting from Pb intoxication was mediated by two different mechanisms. One mechanism is involved inhibitory effects on P450 by Pb at the transcriptional level; the second was heme- dependent, as Pb-mediated inhibition of heme synthesis decreased the heme saturation of P450 and the apo-P450 ratio.

The effect of heavy metals (Cd, Co, Cu, Ni, Pb, and Zn) on 3-methylcholanthrene-induction of cytochrome P4501A and the activity of ethoxyresorufin-O-deethylase (EROD) were investigated in fish hepatoma cells (PLHC-1) by Brüschweiler et al. (1996). The authors reported that all the heavy metals tested had more pronounced effects on EROD activity compared to controls. The inhibitory potency of Pb was reported to be very low compared to cadmium or cobalt. A single treatment of Pb acetate induced hepatic DT diaphorase activity (Sugiura et al., 1993). This induction of hepatic DT diaphorase by Pb acetate has been reported to be decreased with concomitant administration of Dil, a calcium antagonist. Based on these observations, Arizono et al. (1996) suggested that DT diaphorase induction by Pb acetate may occur de novo via protein synthesis mediated by increased cellular calcium. The potential interaction of metals, including Pb, on the induction of CYP1A1 and CYP1A2 by polycyclic aromatic hydrocarbons (PAHs) in human hepatocyte cultures was investigated by Vakharia et al. (2001). Lead nitrate, like other metals such as Cd, Hg, and As, decreased the extent of CYP1A1 and CYP1A2 induction by five different PAHs. The authors concluded from these studies that Pb (5 μM) diminished the induction of CYP1A1 and CYP1A2 in human hepatocytes by ultimately decreasing the levels of CYP1A1 protein that was normally attainable through PAH induction. Korashy and El-Kadi (2004) also investigated similar interactions of metals with aryl hydrocarbon receptor (AHR)-regulated gene expression and enzyme activities in wild-type murine hepatoma cells (Hepa 1c1c7) and AHR-deficient cells (C12). These studies indicated that metals alone (including Pb) did not significantly alter CYP1A1 proteins or activity, or change AHR ligand-induced enzyme activity. There was no change in mRNA levels. Lead, in the presence or absence of AHR ligand, increased the activity of NAD(P)H:quinone oxidoreductase and its mRNA levels.

Phase II Enzymes

A single injection of Pb nitrate (5-10 µM/100 g body wt) was found to increase GST activity levels (Roomi et al., 1986). Additional studies by the same group identified induction of a specific form GST-P by Pb nitrate in rat liver (Roomi et al., 1987). Because a single injection of Pb nitrate decreased phase I and increased phase II hepatic enzymes, these investigators concluded that Pb nitrate treatment initiated a biochemical phenotype similar to carcinogen-induced hepatocyte nodules. Immunohistochemical analysis by the same group reported that Pb nitrate administration resulted in the appearance of GST-P in most of the hepatocytes, an enzyme that is otherwise undetectable in normal rat liver (Columbano et al., 1988; Roomi et al., 1987). On the other hand, Nakagawa (1991) reported inhibition of GST on acute exposure to Pb and that the inhibition of GST followed a reduction in liver GSH levels. Nakagawa (1991) concluded that the depletion of GSH was not necessarily a critical factor in inhibiting GST.

Planas-Bohne and Elizdale (1992) found that acute exposure to Pb nitrate (100 µmol/kg) caused a significant increase in liver and kidney GST activity. Gel electrophoresis analysis to evaluate the contribution of various GST isoforms indicated that enhancement of liver GST activity was predominantly due to induction of GST isoform 7-7 in liver compared to all isoforms in kidney. Liver GST-P isoform was reported to be induced by both Pb acetate and Pb nitrate (Boyce and Mantle, 1993; Koo et al., 1994). This transient induction of GST-P has been regulated at transcription, post-transcription, and post-translational levels. Suzuki et al. (1996) utilized a transgenic approach to investigate the transcriptional regulation of GST-P induced by Pb and identified glutathione S-transferase P enhancer I (GPEI), an enhancer (whose core consists of two AP-1 site-like sequences) located at the 5' flanking region of this gene. The authors demonstrated that GPEI is an essential element in the activation of the GST-P by Pb and that the trans activating factor AP-1 is likely to be involved, at least in part, in the transcriptional activation of the GST-P gene by Pb via the GPEI sequence.

Daggett et al. (1997, 1998) investigated the effect of inorganic and organic Pb on liver GST expression and other phase II detoxifying enzymes in rat liver and kidney. Triethyl Pb chloride (TEL) injection (10 mg/kg body wt) decreased liver GST activity, as well as levels of various other GST isoforms (Daggett et al., 1997), in contrast to significant induction of kidney GST activity, suggesting that a single compound, TEL, had opposite effects on the expression of GST isozymes and indicated the complexity of GST regulation. Similarly, this group also

reported that a single injection of Pb acetate (114 mg/kg body wt) reduced GSH levels, increased production of malondialdehyde (MDA), and did not change the expression of various GST isoforms analyzed, except GST-p1 on repeated injection (Daggett et al., 1998). Similar to studies with TEL, Pb acetate also increased the expression of GST enzyme activity and expression of various isoforms without changing GSH and MDA levels, suggesting that oxidative stress may not be mediating the toxicity in kidney. On the other hand, TEL exposure was found to decrease microsomal estradiol metabolism (Odenbro and Rafter, 1988).
The suppression of GST expression reported by Daggett et al. (1997, 1998) is in contrast to the induction of GST reported by various other groups discussed earlier. Other GSH-dependent enzymes (i.e., GSH peroxidase, GSH reductase) have been found to be suppressed with a simultaneous increase in oxidized GSH (GSSG) and a reduction in GSH/GSSG ratio (Sandhir and Gill, 1995). More detailed information on these and related studies is summarized in Table AX5-10.1.

5.10.1.2 Biochemical and Molecular Perturbations in Lead-Induced Liver Tissue Injury

Oskarsson and Hellström-Lindahl et al. (1989) studied the cellular transport of Pb (^{203}Pb), in rat hepatocytes using dithiocarbamate (DTC). Cells treated with Pb acetate and Pb-DTC lipophylic complex demonstrated increased cytosolic Pb levels compared to Pb alone. This was further evaluated by measuring levels of ALAD. Cells treated with Pb-DTC complex showed rapid and stronger inhibition of ALAD compared to Pb acetate, suggesting that this inhibition was due to increased mobilization of Pb into cells treated with Pb-DTC complex. Another report by the same group, Hellström-Lindahl and Oskarsson (1990), suggested that the increased inhibition of ALAD was due to the release of Pb from the Pb-DTC complex by decomposition. Using the mouse strain with a duplication of the ALAD gene (DBA), Claudio et al. (1997) reported increased accumulation of Pb in this strain by many fold as compared to mice with a single copy of the ALAD gene (C57).

A single injection of Pb nitrate was reported to cause hepatic hyperplasia correlating with hepatic de novo synthesis of cholesterol along with alterations in glucose and lipid metabolism leading to altered serum lipid profiles (Dessi et al., 1984; Pani et al., 1984). Mobilization of hepatic glycogen and altered gluconeogenic enzymes, including differential expression of G6PD, has been reported following Pb exposure (Batetta et al., 1990; Hacker et al., 1990). Chronic Pb

intoxication has also been reported to inhibit gluconeogenic enzymes, alterations that were implicated in Pb bio-transformation rather than liver cell proliferation in Wistar rats (Calabrese and Baldwin, 1992). Although these studies point out to newer directions regarding Pb effects on hepatic carbohydrate metabolism, due to lack of information on blood Pb levels, they have limited value for extrapolation to human exposure scenarios and associated health effect assessment. Increased levels of serum lipid peroxide (LPO) were also observed in rats given SC injection of Pb acetate, supporting similar increased levels of serum LPO in humans exposed to Pb (Ito et al., 1985). These initial studies suggest that alterations in liver intermediary metabolism occur on exposure to Pb with a role for Pb-induced LPO in hepatotoxicity and potential involvement of oxidative stress in Pb toxicity. Limited studies on the hepatic lipid provide peroxidation blood Pb levels in the range of 18 to 35 µg/dL.

Dessi et al. (1990) investigated the role of fasting on Pb-induced hepatic hyperplasia by monitoring the activities of enzymes involved in cholesterol synthesis and the hexose monophosphate shunt and reported that stimulation of these enzymes, even in Pb acetate-treated fasting rats, supported the role of new endogenous synthesis of cholesterol and gluconeogenic mechanisms in Pb-induced hepatic cell proliferation. Chronic exposure to Pb was found to increase the arachidonate/linoleic acid ratio in liver and serum (Donaldson and Leeming, 1984; Donaldson et al., 1985) along with the GSG concentration (McGowan and Donaldson, 1987). As GSH and arachidonate are precursors for peptido-leukotrienes, Donaldson's group investigated the potential effects of dietary Pb on levels of fatty acids, peptido-leukotrienes, and arachidonate/linoleic ratios in chicken fed with diets low in calcium and methionine. These investigations found similar increases in arachidonate/linoelic acid ratio and in GSH levels without bearing on peptido-leukotriene levels. The authors also found the influence of a low calcium and methionine diet on Pb-induced serum fatty acid profiles (Knowles and Donaldson, 1990).

Chronic sublethal Pb exposure (5 ppm Pb nitrate for 30 days) has been found to alter liver lipid profiles in blood and liver tissue of the fresh water fish *Anabas testudineus* (Tulasi et al., 1992). These authors reported significant increases in liver total lipids, cholesterol, and free fatty acids. Tandon et al. (1994) reported that iron deficiency enhanced the accumulation of Pb in liver and kidney and also increased liver calcium levels. Induced expression of metallothionein (MT) in renal and intestine was also observed in iron deficiency. Han et al. (1996) investigated

the effect of Pb burden on weight loss using an energy restriction diet regimen on rats with prior Pb exposure. The authors reported that rats on a substantial weight loss regimen (40% of normal calories) exhibited a significant increase in the quantity and concentration of liver Pb and a decrease in the concentration of other metals (e.g., Ca, Cu, Mg, Zn). The authors concluded that weight loss can increase the liver concentration of Pb, even in the absence of continued exposure. Combined exposure to Pb (70 mg/kg) and Cd (20 mg/kg) in Buffalo rats for 7 weeks was found to alter liver levels of Zn and Cu, with less accumulation of Pb and Cd, compared to individuals exposure to either Pb or Cd alone (Skoczyńska et al., 1993). These authors also reported that a combined exposure regimen interfered with serum lipid profiles (Skoczyńska and Smolik, 1994).

Liu et al. (1997) utilized rat primary hepatocyte cultures to explore the protective effect of Zn-induced expression of metallothionein (MT) in Pb toxicity. These authors found that, in the control cells without prior Zn exposure, most of the Pb was found bound to high-molecular weight proteins in the cytosol, while in the Zn pretreated cells, a majority of Pb bound to MT, indicating a MT-mediated protection against Pb toxicity to hepatocytes. More details about these and related studies are summarized in Table AX5-10.2.

5.10.1.3 Effects of Lead Exposure on Hepatic Cholesterol Metabolism

Lead nitrate-induced hyperplasia or liver cell proliferation involves simultaneous increase in both liver and serum total cholesterol levels. Recent studies have reported various molecular events associated with this process. Induction of gene expression for CYP51 (Lanosterol 14α-demethylase), an essential enzyme for cholesterol biosynthesis, was reported in Pb nitrate-induced liver hyperplasia, although other cytochrome P450 enzymes involved in drug metabolism have been reported as being suppressed, as discussed in earlier sections. This gene has various regulatory elements and its constitutive expression in liver is mediated by sterol regulatory element (SRE) and by the SRE binding proteins-1a, 2, and 1c. Kojima et al. (2002) reported that Pb nitrate induced the expression of CYP51 in the livers of both immature (4-week-old) and mature (7-week-old) rats and that this induction appeared to be mediated by the upregulation of SRE binding protein-2. However, this increased synthesis of cholesterol observed in rat liver was not mediated by endogenous feedback regulation by sterols, as no decrease in serum total cholesterol was observed. To understand the molecular mechanisms

involved in the Pb nitrate-mediated development of hepatic hypercholesterolemia, Kojima et al. (2004) investigated the expression of various enzymes involved in cholesterol homeostasis, including some of the associated transcription factors in male rats exposed to Pb nitrate (100 μmol/kg body wt). The authors reported that Pb nitrate exposure caused a significant increase in liver and serum total cholesterol levels at 3 to 72 h and 12 to 72 h, respectively. The enzymes involved in cholesterol biosynthesis viz. (i.e., 3-hydroxy-3methyglutaryl-CoA reductase, farnesyl diphosphate synthase, squalene synthase, CYP51) were all activated (3-24 h), while the enzymes involved in cholesterol catabolism such as 7α-hydroxylase were remarkably suppressed 3 to 72 h. Figure 5-19 shows the involvement of Pb at various stages of the cholesterol synthesis pathway. The induction of the cytokines interleukin-1α and TNF-α in rat liver prior to the induction of the genes for these synthesis enzymes suggested that Pb nitrate-induced cholesterol synthesis is independent of sterol homeostasis regulation. Following gestational and lactational exposure to Pb acetate (0.05 mg/kg body wt), Pillai and Gupta (2005) reported that the activities of the hepatic steroid metabolizing enzyme 17-β-hydroxy steroid reductase, UDP glucouronyl transferase, and CYP450 levels decreased in rat pups on PND21.

Alterations in the hepatic system of neonates and pups (at PND12 and PND21) after gestational and lactational exposure to Pb acetate (300 mg/L) have been reported by Corpas et al. (2002a). The authors found significant reductions in the liver weight of pups and in hepatic glycogen that correlated with increased blood glucose levels. The authors also reported reductions in liver protein, lipid levels, and alkaline and acid phosphatase activities but did not find any gross structural alterations in liver tissue. These and other studies are summarized in Table AX5-10.3.

5.10.1.4 Effect of Lead on Hepatic Oxidative Stress

Although several mechanisms have been proposed to explain Pb toxicity, no mechanism has been defined explicitly. Recent literature on Pb toxicity suggests oxidative stress as one of the important mechanisms of toxic effects of Pb in liver, kidneys, brain, and other organs. Schematic representation of the various mechanisms by which Pb induces lipid peroxidation is shown Figure 5-20. Lead toxicity to the liver has been found to be associated with significant accumulation of Pb in the liver. This results in the accentuation of lipid peroxidation with concomitant inhibition of antioxidant enzymes (i.e., SOD, catalase, GSH peroxidase, GSH

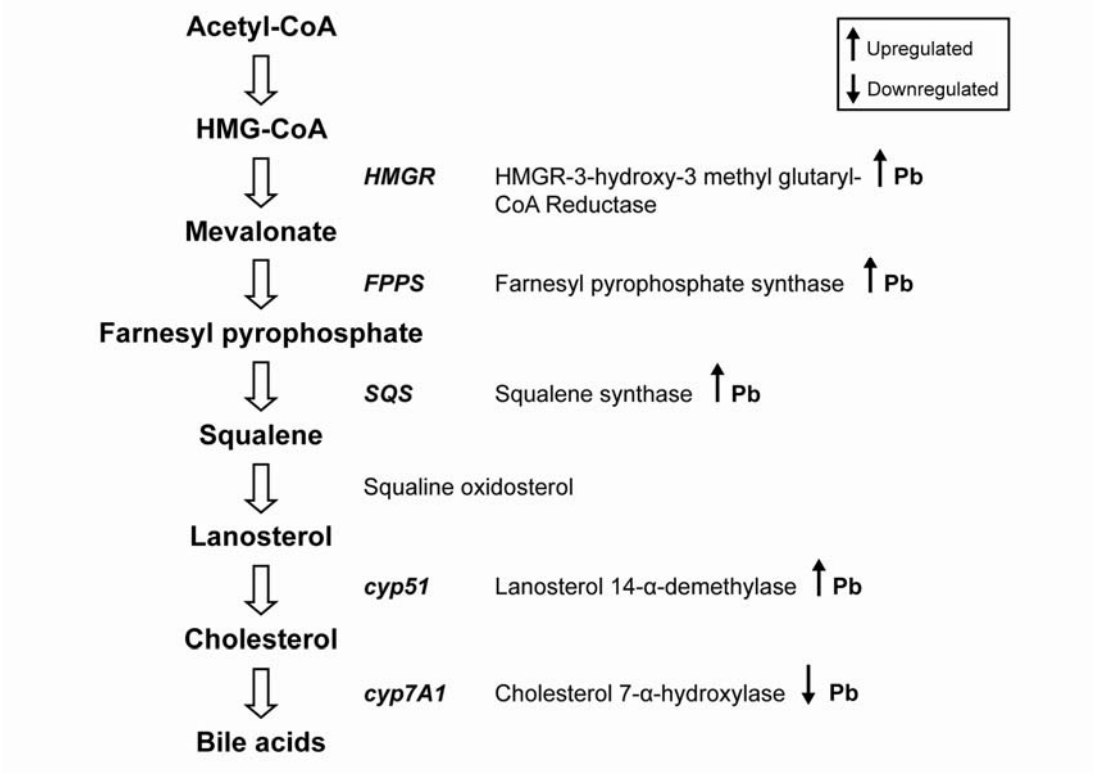

Figure 5-19. Flow diagram indicating the lead effects on the cholesterol synthesis pathway.

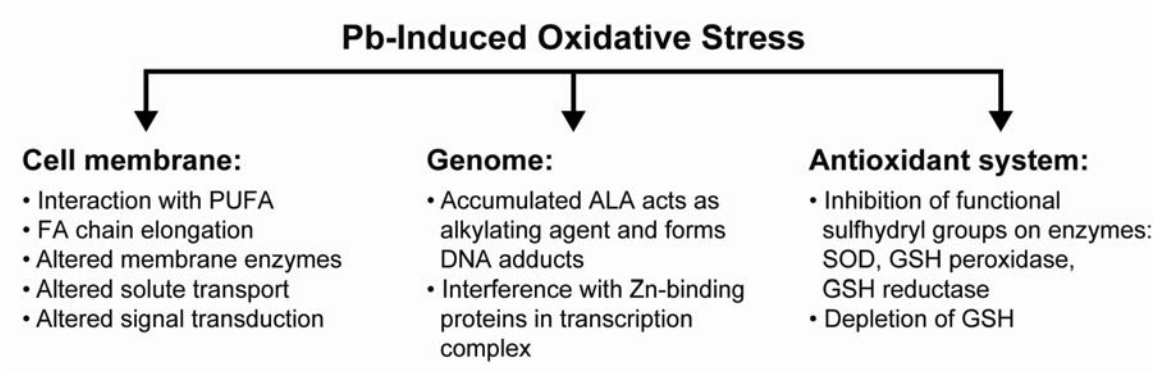

Figure 5-20. Schematic diagram illustrating the mode of lead-induced lipid peroxidation.

reductase) and a simultaneous increase in GSSG with a reduction in GSH/GSSG ratio (Sandhir and Gill, 1995; Aykin-Burns et al., 2003). However, Furono et al. (1996) studied the potential of various redox-active metals to induce LPO in normal and alpha-linolenic acid-loaded rat hepatocytes and suggested that Pb ions were not capable of inducing lipid peroxidation in such hepatocytes.

The currently approved clinical intervention method is to give chelating agents that form a soluble complex with Pb and remove the same from Pb-burdened tissues. The details of these studies are provided in Annex Table AX5-10.4.

5.10.1.5 Lead-Induced Liver Hyperplasia: Mediators and Molecular Mechanisms

The biochemical and molecular events associated with Pb-induced hyperplasia has been accumulating in the scientific literature. Lead nitrate, a known mitogen, is also considered to be a carcinogen that induces liver cell proliferation in rats without any accompanying liver cell necrosis. It has been recognized that this proliferation is a transient process and that apoptosis plays a major role in the regression of Pb nitrate-induced hepatic hyperplasia (Nakajima et al., 1995). Columbano et al. (1996) studied the cell proliferation and regression phases by apoptosis in Wistar male rat liver by monitoring the incorporation of tritiated thymidine as a marker for increased DNA synthesis. These studies demonstrated the production of Pb-induced proliferation 3 days after a single injection of Pb nitrate with complete regression of hyperplasia seen after 15 days. The authors suggested that the apoptosis process observed in the regression phase also involved newly initiated hepatocytes. On the other hand, Dini et al. (1999) reported the regressive or involutive phase as beginning 5 days post single injection of Pb nitrate. Apostoli et al. (2000) evaluated the proliferative effects of various Pb salts (i.e., Pb acetate, Pb chloride, Pb monoxide, Pb sulfate) using liver-derived REL cells. These authors reported that all the Pb compounds tested showed dose- and time-dependent effects on the proliferation of REL cells. Unlike other tumor promoters, Pb compounds did not exhibit effects on cell junctional coupling. Liver hyperplasia induced by Pb nitrate has been shown to demonstrate sexual dimorphism in all phases of the proliferation as well as in apoptosis (Tessitore et al., 1995). Biochemical changes associated with liver hyperplasia in the intermediary metabolic pathways were discussed in earlier sections of this chapter; the present discussion focuses on other molecular characteristics of this process. As the numerous molecular networks involved in both

the proliferation and apoptosis processes have many common mediators and pathways, it is very difficult to provide a discussion without an overlap.

DNA hypomethylation has been recognized to play a major role in the proliferation of cells in regenerating and in hepatic pre-malignant lesions when compared to normal non-dividing liver cells. A single dose of Pb nitrate (75 μM/kg body wt) has been found to cause extensive hypomethylation in rat liver (Kanduc et al., 1991). Additional investigations from the same group reported that this hypomethylation status of liver DNA by Pb nitrate changed significantly with age and exhibited liver cell specificity (Kanduc and Prisco, 1992).

Investigations of cell cycle-dependent expression of proto-oncogenes in Pb nitrate (10 μM/100 g body wt)-induced liver cell proliferation by Coni et al. (1989) showed that peak DNA synthesis occurred at 36 h after a single injection of Pb nitrate. In addition to DNA synthesis, induced expression of c-fos, c-myc, and c-Ha-ras oncogenes was also observed in rat liver tissue. Additional studies by the same group reported that Pb nitrate-induced liver hyperplasia involved an increased expression of c-jun in the absence of c-fos expression (Coni et al., 1993). The induced expression of c-myc persisted up to 40 h post Pb nitrate exposure. Lead nitrate-induced liver proliferation and DNA synthesis, as monitored by 5-bromo-2-deoxyuridine immunohistochemistry, led to DNA labeling in a few hepatocytes (Rijhsinghani et al., 1993). The observed DNA synthesis appeared to be due to the increased activity and expression of DNA polymerase-α observed at 8 h postexposure to a single injection of Pb nitrate (Menegazzi et al., 1992). Along with DNA synthesis, poly (ADP-ribose) polymerase was also induced by Pb nitrate (Menegazzi et al., 1990). Differential activation of various PKC isoforms, downregulation of PKC-α, and marked activation of PKC-ϵ in Pb nitrate-mediated liver hyperplasia suggested the involvement of these PKC enzymes in DNA synthesis and related signal transduction pathways (Tessitore et al., 1994; Liu et al., 1997).

Coni et al. (1992) reported the proliferation of normal and pre-neoplastic hepatic cells treated with the plasma derived from male Wistar rats treated with a single injection of Pb nitrate; this was the first report on the secretion of biological cell proliferation signals in the liver after Pb nitrate treatment. These authors reported that DNA synthesis was detected as early as 30 min and persisted up to 5 days after Pb nitrate exposure. This observation has opened up the inquiry into the involvement of various growth factors and other biological mediators in hepatic hyperplasia. Shinozuka et al. (1994) investigated the expression of various growth factors (i.e.,

hepatocyte growth factor, TGF-α, TGF-β) in rat liver after a single injection of Pb nitrate (100 μM/kg body wt) and reported the involvement of these growth factors in liver cell proliferation. Additional studies by this group to observe LPS sensitivity in rats given Pb nitrate reported that animals given a single injection of LPS up to 100 μg survived, whereas in the presence of Pb nitrate, they tolerated only 6 μg of LPS, indicating that Pb nitrate may sensitize the animals for LPS toxicity.

Earlier studies by Honchel et al. (1991) reported that coexposure of rats to Pb acetate (15 mg/kg) and LPS or TNF showed markedly increased serum levels for various liver injury parameters. They concluded that Pb may potentiate liver toxicity by LPS via a TNF-mediated pathway. The role of TNF-α in Pb nitrate-induced liver cell proliferation was further investigated by (Ledda-Columbano et al., 1994) who demonstrated the inhibition of Pb nitrate-induced cell proliferation by pretreatment with dexamethasone, an inhibitor of TNF-α expression. Additional studies by the same group evaluated the liver cell specificity in Pb nitrate-induced cell proliferation (Shinozuka et al., 1996). They monitored the incorporation of 5-bromo-2-deoxyuridine by immunohistochemical analysis on rat liver as induced by Pb nitrate and TNF-α and observed 5-bromo-2-deoxyuridine incorporation in hepatocytes and non-parenchymal cells (i.e., Kupffer cells, endothelial cells, periportal nondescript cells), confirming that Pb-induced liver cell proliferation was mediated by TNF-α. Kubo et al. (1996) used various TNF-α inhibitors to further confirm the role of TNF-α in Pb nitrate-induced hepatocyte proliferation. Menegazzi et al. (1997) reported that Pb nitrate-induced proliferation involved the induction of iNOS along with TNF-α and that appeared to be mediated by a strong, prolonged activation of NFκB but not activator protein-1 (AP-1). Nemoto et al. (2000) investigated the potential role of neurotrophins and their receptors in Pb nitrate-induced hepatic hyperplasia. The expression profile of TNF-α, neurotrophins (i.e., nerve growth factor, brain-derived neurotrophic factor neurotrophin-3 and (their receptors), tyrosine kinase receptor (Trk) and neurotrophin receptor (p75NTR) were investigated in liver tissue after a single injection of Pb nitrate (100 μM/kg body wt). The Pb nitrate-induced increased expression of TNF-α preceded the expression of the neurotrophins and their receptors. Based on these results, the author's suggested that neurotrophins and neurotrophin receptors are involved in mediating mitogenic signals related to hepatic hyperplasia.

The regression phase of Pb-induced liver hyperplasia appears to be mediated by OS. As discussed earlier, this process involves LPO and other cytokine mediators, including TNF-α. Sieg and Billings (1997) reported that Pb potentiated cytokine-induced OS, producing a significant decline in intracellular ATP concentration in mouse hepatocyte culture studies. The authors suggested that cytotoxic interaction between Pb and cytokines (e.g., TNF-α and IFN) may be mediated by oxidative DNA damage resulting from OS. The potential role OS along with TNF-α has been implicated in the apoptosis of hepatocytes by Milosevic and Maier (2000). Using freshly isolated cultures of hepatocytes and Kupffer cells and their co-culture system exposed to Pb acetate (2-50 μM) and LPS (0.1-1000 ng/mL), the authors reported that, in the co-culture system, the Pb-LPS-induced release of TNF-α from the Kupffer cells, increased nitric oxide levels by 6-fold and downregulated the acute phase protein, albumin, in hepatocytes. From these observations the authors concluded that Pb-induced Kupffer cell-derived signals promoted the toxicity of Pb in hepatocytes, resulting in hepatocyte death by proteolysis. The importance of the Kupffer cells role in Pb nitrate-induced heptatocyte apoptosis was further demonstrated (Pagliara et al., 2003a,b). These authors reported that in vivo hepatic apoptosis including oxidative response induced by Pb nitrate, was prevented by pretreatment with gadolinium chloride, a Kupffer cell toxicant that specifically suppresses Kupffer cell activity. When treated hepatocytes were exposed in vitro to Pb nitrate, hepatocyte apoptosis was not observed. On the other hand, hepatocyte apoptosis was evident when the hepatocytes were incubated with culture medium derived from Kupffer cells that had been exposed to Pb nitrate. Based on these studies, the authors concluded that heptocyte apoptosis was potentiated by soluble factors secreted by Pb-exposed Kupffer cells. The role of activated Kupffer cells, macrophages, and TNF-α in chemical-induced hepatotoxicity is presented schematically in Figure 5-21.

Dini et al. (1993) investigated the expression of asialoglycoprotein receptors on the surface of hepatocytes and galactose-specific receptors of non-parenchymal cells during the apoptic phase of Pb-induced hepatic hyperplasia. A significant increase in asialoglycoprotein receptor expression in hepatocytes coincided with massive apoptosis. Later studies from this group demonstrated that sinusoidal liver cells predominantly phagocytosed the Pb nitrate-induced apoptic hepatic cells and concluded that this process appeared to be mediated by the cell surface carbohydrate receptors (i.e., mannose and galactose receptors) (Ruzittu et al., 1999).

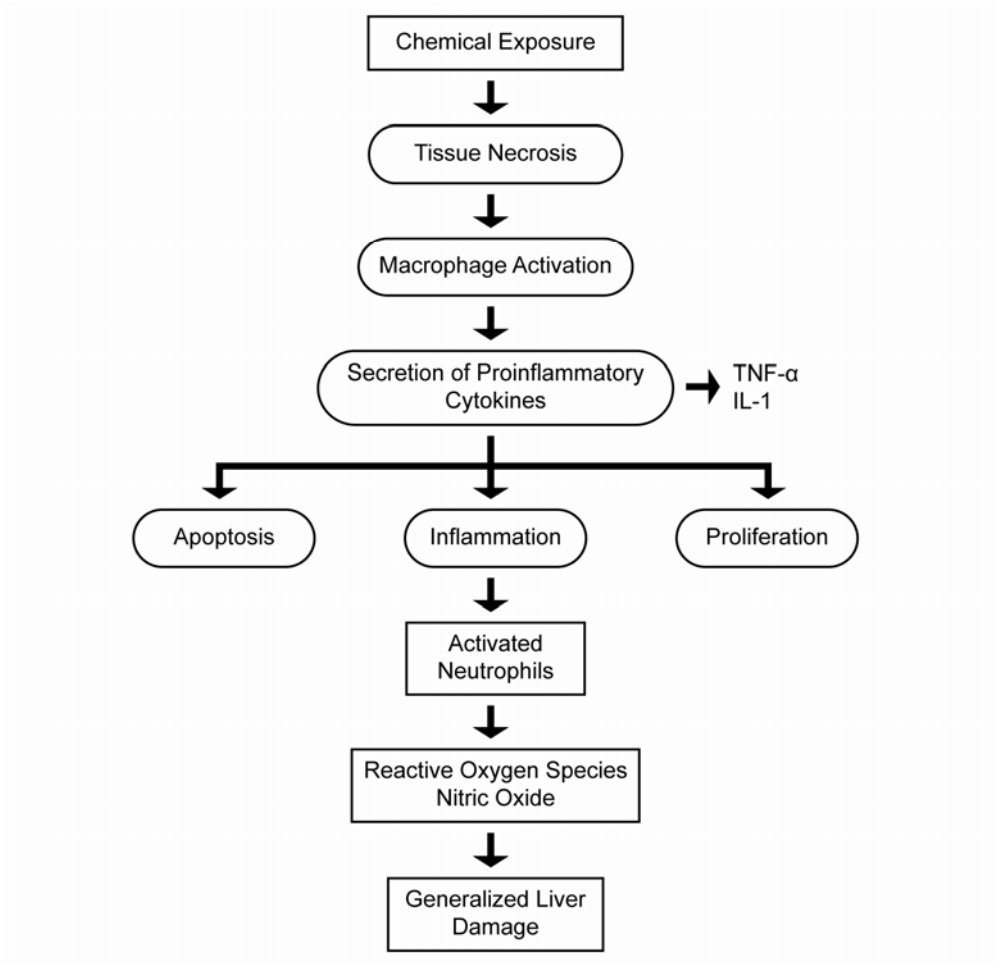

Figure 5-21. Hypothesis of chemical-induced liver injury generated primarily on the basis of different types of inhibitors.

Pretreatment of rats with gadolinium chloride, a kupffer cell toxicant, was also found to abolish the altered expression of galactose receptors (Pagliara et al., 2003b).

The role of glucocorticoid-mediated signal transduction in the hepatotoxicity of Pb was evaluated by Heiman and Tonner (1995), using H4-IIE-C3 hepatoma cells (HTC). Acute exposure of cells to Pb (300 nM^{-1} or 10 μM) was found to inhibit processes involved inglucocorticoid-mediated enzyme induction (e.g., tyrosine aminotransferase activity) in a dose-dependent manner both at the transcriptional and translational level, without altering glucocorticoid receptor binding characteristics. Tonner and Heiman (1997) also reported Pb-induced hepatotoxicity by glucocorticoid-mediated signaling and its involvement in the interference with calcium-mediated events as well as the differential modulation and

translocation of protein kinase isoforms α and β into the nucleus. More information on these and other related studies is summarized in Table AX5-10.5.

5.10.1.6 Effects of Lead on Liver Heme Synthesis

Effects of Pb on heme metabolism have been extensively investigated in major target tissues such as liver and erythrocytes. Section 5.2 described Pb effects on heme synthesis, with particular relevance to erythrocytes. The effects of Pb on heme synthesis in the liver and the role of chelation therapy in this process are discussed in this section.

Fifteen percent of heme is produced in the liver. Heme metabolism in the liver is an essential component of various cytochrome P450s that participate in cellular redox reactions and xenobiotic detoxification pathways in the liver tissue and, hence, heme plays a vital role in liver function (Jover et al., 1996). Due to the important and critical role of heme in liver function, Pb-induced effects on hepatic heme metabolism are discussed below.

Initial studies on the effects of Pb nitrate on hepatic heme biosynthesis were reported by Lake and Gerschenson (1978) using the rat liver cell line (RLC-GAI). The effects of various organic metal compounds on ALAD activity have been studied by Bondy (1986). The authors reported that triethyl Pb chloride has the same potency as Pb nitrate in inhibiting ALAD both in vitro and in vivo, with liver and blood ALAD exhibiting similar sensitivities to Pb compounds. By measuring the conversion of ALA into heme, these authors showed that heme biosynthesis was inhibited by Pb in a dose dependent manner. Using a lipophilic complex of Pb acetate + DTC to increase the cellular uptake of Pb, Osksarsson et al. (1989) demonstrated the inhibition of ALAD activity in primary rat hepatocytes cultures. Lead-acetate has been reported to inhibit ALAD activity in rabbit liver tissue without any effect on delta-aminolevulinic acid synthase (ALAS) activity (Zareba and Chemielnicka, 1992). Exposure to Pb (500 ppm) in drinking water did not inhibit hepatic ALAS, but did inhibit ALAD activity in mice (Tomokuni et al., 1991). Exposure to Pb acetate (20 mg/kg body wt for 3 days) has been reported to decrease hepatic ALAD and uroporphyrinogen activity (Satija and Vij, 1995). These authors also reported that IP injection of zinc (5 mg/kg body wt for 3 days) conferred protection against Pb acetate effects in liver tissue.

Effects of Pb on hepatic porphyrins, intermediate metabolites of heme metabolism, were investigated by few researchers. Quíntanilla-Vega et al. (1995) reported that 3T3-hepatocyte

cultures, when incubated with a micromolar concentration of Pb acetate increased cellular porphyrin content and excretion. This increased porphyrin production may have been due to an accumulation of protoporphyrin and coproporphyrin, as in coproporphyrinuria, a well-characterized sign of Pb intoxication (Ichiba and Tomokuni, 1987; Zareba and Chemielnicka, 1992). Dietary supplementation of selenium and monensin increased Pb-induced accumulation of prophyrins in chicken liver (Khan and Szarek, 1994). Species-specific differences in the effects of Pb on protoporphyrins were reported by Jacobs et al. (1998). These authors investigated the effect of Pb on zinc protoporphyrin synthesis in cultured chick and rat hepatocytes and observed decreased levels of protoporphyrin in rat hepatocytes, but no effect on chick hepatocytes. Santos et al. (1999) also reported Pb-induced derangements (including porphyrin metabolism) in rat liver heme metabolism, but these effects were far less severe than those observed in erythrocytes. Their investigations on the effect of chronic alcoholism on Pb effects in hepatic heme metabolism suggested no potentiation by alcohol.

Transferrin (TF) is the major iron-transport protein in serum and other biological fluids. Transferrin can also has the capacity to transport other metals. Lead was found to inhibit TF endocytosis and transport of iron across the cell membrane of rabbit reticulocytes (Qian and Morgan, 1990). The effect of Pb on TF gene expression was investigated by Adrian et al. (1993) using a transgenic mouse with the human TF gene. They found that Pb suppressed the expression of TF transgene in mouse liver at the transcriptional level; however, the same dose of Pb did not inhibit mouse endogenous hepatic TF gene expression. Lead exposure was also found to inhibit recombinant TF expression in human hepatoma hepG2 cells. Other studies by the same group found that Pb exposure suppressed the expression of endogenous TF in HepG2 cells (Barnum-Huckins et al., 1997). These authors further suggested that Pb effects on hepatic TF levels may also interfere with iron metabolism in humans. (See Annex Table AX5-10.6 for more information on these and related studies.)

5.10.2 Gastrointestinal System and Lead Absorption

Lead enters the body by many routes, but primarily via the GI tract. The intestinal epithelium serves as one of the body's primary interfaces with the outside world. The transporting epithelia in the small intestine are characterized by layers of anatomically and biochemically polarized cells that are connected to each other by tight junctions and resting on a

basement membrane. Classically, the intestinal epithelium is thought of primarily as a barrier, but it also is a highly reactive barrier. Even modest perturbations in its functions may lead to diarrhea, constipation, malnutrition, dehydration, and infectious diseases (i.e., ulcerative colitis, collectively referred as chronic intestinal inflammatory diseases) (Gewirtz et al., 2002). Abdominal colic and constipation are symptoms of Pb poisoning, but its mechanism is not fully understood. Studies have been carried out in the past decade to increase our understanding of the fundamental mechanism(s) in order to extrapolate the experimental observations to human health effects.

One key factor required to access Pb-related risks is the understanding and quantification of bioavailability. Detailed discussions on the bioavailability of Pb, methodological approaches for bioavailability measurements, bioavailability and speciation, etc. are discussed in detail in Section 4.2. This section is primarily focused on the gastrointestinal absorption of Pb with relevance to animal studies and in vitro test systems of intestinal origin.

The intestinal absorption of Pb is influenced by a variety of factors, including the chemical and physical forms of the element, age at intake, and various nutritional factors. Gastrointestinal absorption of Pb is thought to occur primarily in the duodenum. In the isolated rat intestine, absorption, and, in particular, serosal Pb transfer activity (net transfer of Pb from the small intestine lumen across the epithelium and into the serosal space) is highest in the duodenum. The mechanisms of absorption may involve active transport and/or diffusion through the intestinal epithelial cells. Both saturable and non-saturable pathways of absorption have been inferred from the studies in different animal models, although the understanding of the former is slightly more robust (Diamond et al., 1998).

Transport of Pb as a complex with proteins via endocytosis or as a complex with amino acids are postulated as possible mechanisms. Direct evidence for transport of an organic Pb complex has not been provided, but it seems possible.

In the cell, Pb interacts with a variety of intracellular ligands, including calcium-binding proteins and high-affinity Pb-binding proteins. Transfer across the cell or basolateral membrane (or both) involves a mechanism(s) that may be sensitive to vitamin D and iron status. Alternate transport mechanisms via a Ca^{2+}-Na^{+} exchanger, independent of regulation by vitamin D, are also possible.

5.10.2.1 Lead and In Vitro Cytotoxicity in Intestinal Cells

In vitro cytotoxicity of metal salts for 48 h was determined in the intestinal epithelial cell line I-407 by Keogh et al. (1994). The investigations identified rank order cytotoxicity in terms of LC_{50} values: $HgCl_2$ (32 μM) > $CdCl_2$ (53 μM) > $CuCl_2$ (156 μM) > Ti_2SO_4 (377 μM) > Pb $(NO_3)_2$ (1.99 mM). Further studies using a noncytotoxic concentration of butathione sulphoxamine pretreatment for GSH depletion revealed that the cytotoxicity of Pb was unaffected by GSH depletion (see Table AX5-10.7).

5.10.2.2 Alterations in Intestinal Physiology and Ultrastructure

Karmakar et al. (1986) investigated the pathologic alterations that occur in the intestine, liver, and kidney of Pb-intoxicated rats upon short-term exposure to sublethal doses of Pb (44 mg/Kg body wt) and reported degeneration of intestinal mucosal epithelium leading to potential malabsorption.

The effect of low-concentration Pb acetate (0.1%) on the jejunal ultrastructure was studied by Tomczok et al. (1988) in young male rats. The studies revealed that the villi of jejunum of rats exposed to Pb for 30 days had a rough appearance on the surface, which could be associated with a distortion of glycocalyx layer. Areas of extensive degenerative lesions were also observed on the surface of most villi on the 60th day of exposure. All intestinal epithelial cells exhibited various degrees of glycocalyx disturbance, indicating that pronounced toxic effects of Pb were related to modifications of the biochemical properties of the surface coat of the cells. These authors also reported the appearance of goblet cells and of Pb deposition along the goblet cell membrane in blocks of tissue along the border between duodenum and jejunum. Continued treatment up to 60 days resulted in mucus droplets in the cytoplasm of goblet cells, along with deposition of silver salts indicative of Pb in these cells. These results demonstrated the significance of goblet cells in Pb detoxification.

In another study on the ultrastructure of rat jejunum exposed to Pb acetate (100 mg/kg body wt/day), Tomczok et al. (1991) found that 30-day treatment resulted in numerous small, rough-membraned vesicles and dilated golgi complexes in the cytoplasm. Continued treatment for 60 days resulted in vacuolated cytoplasm associated with the golgi complexes, rough-membraned vesicles, and dilated cisternae. Also, the surface of the intestinal epithelial cell microvilli showed evidence of Pb deposition, as evidenced by Timm sulfide silver reaction sites.

5.10.2.3 Intestinal Uptake and Transport

Infants are a particularly susceptible population for Pb toxicity, possibly due to the immaturity of the digestive tract, feeding pattern, or source of Pb. To investigate these aspects, Henning's group (Beach and Henning, 1988; Henning and Cooper, 1988) carried out a series of experiments using suckling rat pups and reported that Pb in rat and bovine milk and infant milk formula was primarily associated with casein micelles. Casein-bound Pb may be the most common form of Pb presented to the small intestine (Beach and Henning, 1988). Other studies by this group investigated potential differences in the mechanisms when Pb was presented in ionic or milk-bound form, using ^{203}Pb as a tracer. These studies clearly showed that when ^{203}Pb was administered intragastrically as a soluble salt, it was primarily accumulated in the duodenum, regardless of dose or vehicle used. In contrast, substantial accumulation of ^{203}Pb was found in the ileal tissue following Pb administration in milk. These studies clearly indicated strikingly different patterns in the intestinal accumulation of ionic and milk-bound Pb and suggest a greater toxicity for Pb in drinking water compared to Pb ingestion via milk (Henning and Cooper, 1988).

Dekaney et al. (1997) investigated the uptake and transport of Pb using intestinal epithelial cells (IEC-6). The authors observed that Pb accumulation in Pb-exposed (5-10 μM) IEC-6 cells was time- and dose-dependent up to 1 h and that reduction of the incubation temperature significantly reduced the total cellular Pb content of IEC-6 cells. Simultaneous exposure to Zn resulted in decreased cellular Pb content compared to cells exposed to Pb only. Exposure of cells to ouabin or sodium azide has been found to increase Pb accumulation in the cells compared to cells treated with Pb (5 μM) alone. These studies clearly demonstrate that Pb transport in IEC-6 cells is time- and temperature-dependent, involves the presence of sulfydryl groups, and competes with the uptake of Zn.

Lead speciation and transport across intestinal epithelium in artificial human digestive fluid (chyme), both in vivo and in vitro, in Caco-2 cells were evaluated by Oomen et al. (2003). In vivo studies indicated that in chyme, Pb-phosphate and Pb-bile complexes are important fractions. The metal ions dissociated from these complexes can subsequently be transported across the intestinal epithelium or they may traverse the intestinal membrane. In vitro studies, on the transport of bioaccessible Pb across the intestinal epithelium in Caco-2 cells exposed to diluted artificial chyme for 24 h, indicated that 3% of the Pb was transported across the cell

monolayer. Lead associated with cells in a linear relationship to the total amount of Pb in the system. Bile levels were not found to affect the fraction of Pb associated with the cells. The free Pb^{2+} concentration in chyme was negligible. Extrapolating these results to the in vivo situation, the authors concluded that Pb species other than the free metal ion may have contributed to the Pb flux towards the cells, possibly involving the dissociation of labile Pb species, such as Pb-phosphate and Pb-labile complexes and the subsequent transport of the released free metal ions toward the intestinal membrane.

5.10.2.4 Alterations in Gastrointestinal Motility/Gastrointestinal Transit and Function

The effect of Pb on contractility of rat duodenum was determined in vivo in rats given an oral dose of Pb acetate (44 mg/kg per day, Pb as 53 mM/L for 4 weeks) to investigate the possible mechanisms associated with Pb-induced abdominal colic and constipation (Karmakar and Anand, 1989). Deodenal motility and the amplitude of contractility of rat duodenum were decreased significantly in the Pb-exposed rats, leading the authors to conclude that there was a fundamental change in the contractility of the intestinal tract due to Pb intoxication.

Chronic Pb ingestion through drinking water (2-5 mg/mL, Pb acetate for 55 days) caused a 20-fold increase in urinary excretion of D-ALA and an increase in blood Pb level (80 µg/dL), without any perturbations in propulsive motility of guinea pig colon (Rizzi et al., 1989). On the other hand, Lawler et al. (1991) observed no changes in gastric contractions during ingestion in red-tailed hawks exposed to Pb acetate (0.82 or 1.64 mg/kg body wt for 3 weeks). This low level of exposure has also been found to have no bearing on the regular passing of pellets of undigested material. Shraideh (1999) studied the effect of triethyl Pb chloride on the rhythmic and peristalitic contractile activity of ileum isolated from Swiss mice. These authors observed no significant effect below 40 µM of TEL, while higher concentrations (40-120 µM) caused changes in contraction rhythm. These studies also reported that TEL above 120 µM induced irreversible changes in the ileal contractile activity. These and related studies are summarized in Table AX5-10.8.

5.10.2.5 Lead, Calcium, and Vitamin D Interactions in the Intestine

The complex biological interactions between Pb and calcium have been recognized and demonstrated in virtually every type of tissue. Studies of high-affinity Pb binding to intracellular

calcium receptors and transport proteins, as well as the involvement of Pb in calcium-activated and calcium-regulated processes, have added to our understanding of the effects of Pb on biological processes at the cellular level. The intestinal absorption of Pb is influenced by a variety of factors, including chemical and physical forms of the element, age at intake, and various nutritional factors. Work dating back to the 1940s established that the deposition of Pb in bone and soft tissue significantly increases under conditions of dietary calcium and phosphorus deprivation or by the administration of vitamin D to rachitic animals. Later, in the 1970s, it was demonstrated that dietary calcium status was a major contributing factor determining relative susceptibility to Pb intoxication.

Fullmer's group (Fullmer and Rosen, 1990; Fullmer, 1991, 1992, 1997) carried out a series of studies to investigate the potential interaction between calcium and Pb in the ingestion and intestinal absorption of Pb. Various parameters, such as absorption kinetics for Ca and Pb, activity of alkaline phosphatase, expression of the clabindin D gene, and the potential role of endocrine function in this interaction (as assessed by cholecalciferol and its active hormonal form, 1,25-dihydroxycholecalciferol levels) were investigated. Fullmer and Rosen (1990) observed that chicks fed with low (0.5%) and adequate (1.2%) dietary calcium and exposed to Pb (0-0.8%) exhibited differential effects on intestinal Ca absorption depending on their dietary Ca status. In the chicks fed a low-calcium diet, Pb inhibited intestinal Ca absorption and calbindin D and alkaline phosphatase synthesis in a dose-dependent fashion. On the other hand, chicks fed the normal diet, showed no inhibition of Ca absorption. Based on these results, the authors postulated that Pb-induced alterations in intestinal Ca absorption may involve cholecalciferol and the endocrine system. In an extension of this study using young growing chicks, Fullmer (1991) observed similar results in 2-week Pb-exposed, but not in 1-week exposed, chicks.

As dietary Ca deficiency is associated with a marked increase in the body burden of Pb and in the susceptibility to Pb toxicity during chronic ingestion, Fullmer (1992) examined the effects of vitamin D supplementation on intestinal Pb and Ca absorption. When vitamin D-deficient chicks received physiologic amounts of vitamin D (0.1mg/day), intestinal ^{203}Pb and ^{47}Ca absorption rates were elevated by 4- and 8-fold, respectively. Along with this, calbindin D and alkaline phosphatase activities were also significantly elevated. Ingestion of even the highest level of Pb (0.8 %) during the repletion phase had no effect on intestinal Ca absorption. To further understand the Pb-Ca interactions and the potential involvement of vitamin D on

intestinal absorption, Fullmer (1997) evaluated serum levels of 1,25-dihydroxyvitamin D. Lead ingestion and Ca deficiency alone, or in combination, generally increased serum 1,25-dihydroxyvitamin D levels over most of the ranges of Pb or Ca studied. However, in severe Ca deficiency, Pb ingestion resulted in marked decreases in serum 1,25-dihydroxyvitamin D, intestinal Ca absorption, and calbindin D mRNA. From these studies using response surface models, Fullmer (1997) concluded that the interactions between Pb and Ca were mediated via changes in circulating 1,25-dihydroxy vitamin D hormone, rather than via direct effects on the intestine.

Similar to Ca deficiency, iron deficiency has also been found to increase intestinal absorption of Pb, as indicated by increased blood and kidney Pb levels in iron-deficient rats exposed to dietary Pb; but the mechanistic details are not known (Crowe and Morgan, 1996). These and other related studies are summarized in Table AX5-10.9.

5.10.2.6 Lead and Intestinal Enzymes

Differential effects of Pb on intestinal brush border enzyme activity profiles were reported by Gupta et al. (1994). Across a concentration range of 0.5-6.0 mM, Pb acetate was found to significantly inhibit Ca-Mg-ATPase, g-glutamyl transpeptidase, and acetylcholinesterase activities in a dose-dependent manner without effects on alkaline phosphatase.

Cremin et al. (2001) investigated the effects of oral succimer on the intestinal absorption of Pb in infant rhesus monkeys. These studies indicated that chelation therapy with DMSA for two successive 19-day periods significantly decreased GI absorption of Pb and increased urinary excretion of endogenous lead (see Table AX5-10.9).

5.10.3 Summary

Extensive in vivo and in vitro experimental evidence has accumulated over the past 20 years and increased our understanding of the potential toxic effects of Pb in the hepatic system. These studies ranged from simple biochemical studies to molecular characterizations of the induction of drug-metabolizing enzymes, liver hyperplasia, and the protective effects of chelation therapy.

- Rat liver microsomal cytochrome P-450 levels were found to decrease with a single dose exposure of Pb nitrate. Inhibition of both constitutive and induced expression of microsomal P450 A1 and A2 activity occurred. Simultaneous induction of the activities

of phase II drug metabolizing enzymes with decreased phase I enzymes with single exposure to Pb nitrate suggests biochemical properties similar to hepatic nodules.

- Newer studies examined the induction of GST-P at both transcriptional and translational levels using in vitro systems and indicated a role for Pb nitrate and Pb acetate in the induction process. On the other hand, triethyl Pb compounds have been found to suppress the activity of various GST isoforms.
- Studies on Pb-induced liver hyperplasia demonstrated de novo synthesis of cholesterol, alterations in the gluconeogenic mechanism, as well as DNA hypomethylation and subsequent changes in the expression of protooncogenes.
- Lead-induced alterations in cholesterol metabolism appear to be mediated by the induction of several enzymes related to cholesterol metabolism and the decrease of 7 α-hydroxylase, a cholesterol catabolizing enzyme. This regulation of cholesterol homeostasis is modulated by changes in cytokine expression and related signaling.
- Studies using an inhibitor to block TNF-α have clearly demonstrated TNF-α as one of the major mitogenic signals that mediate Pb nitrate-induced liver hyperplasia. Lead-induced hyperplasia also appears to be modulated by neurotrophins and their receptors.
- In vitro co-culture systems with Kupffer cells and hepatocytes suggested liver cell apoptosis is mediated by Kupffer cell-derived signals and Pb-induced oxidative stress.
- Newer experimental evidence suggests that Pb-induced alterations in liver heme metabolism involves perturbations in ALAD activity, and porphyrin metabolism, alterations in Transferrin gene expression, and associated changes in iron metabolism.
- Limited experimental evidence on the role of weight loss on liver Pb burden in exposed animals indicate that liver Pb content increases even in the absence of prolonged continued exposure.
- Gastrointestinal absorption of Pb is influenced by a variety of factors, including chemical and physical forms of the element, age at intake, and various nutritional factors. The degeneration of intestinal mucosal epithelium leading to potential malabsorption and alterations in the jejunal ultrastructure (possibly associated with distortion of glycocalyx layer) have been reported in the intestine of Pb-exposed rats.
- Lead in rat and bovine milk and, also, infant milk formula was demonstrated to be primarily associated with caseine micelles.
- Tracer studies using ^{203}Pb indicated that intragastric administration of Pb as a soluble salt resulted in Pb primarily accumulating in the duodenum, regardless of dose or vehicle used, whereas Pb from milk was found to be taken up by ileal tissue. Studies also suggested Pb ingestion through water was more toxic than ingestion through milk.
- Lead induced decreases in duodenal motility and amplitude of contractility of the intestinal tract have been reported for rats.
- Nutritional studies using various levels of Pb, Ca, and vitamin D in the diet indicate competition of Pb with Ca absorption. Supplementation with vitamin D has been

reported to enhance intestinal absorption of Ca and lead. Physiological amounts of vitamin D administered to vitamin D-deficient rats resulted in elevated Pb and Ca levels. In the case of severe Ca deficiency, Pb ingestion results in a marked decrease in serum 1,25-dihydroxy vitamin D.

Overall, our understanding of Pb effects on hepatic and gastrointestinal systems using in vitro cell culture models and in vivo animal models has increased greatly compared to the 1986 AQCD. Significant insights have emerged regarding the role of Pb in hepatic cholesterol synthesis, the role of inflammation in Pb-induced hepatotoxicity, and the contribution of newer chelation therapy in the amelioration of Pb-induced oxidative burden. Similarly, our knowledge has greatly enhanced as to the absorption, transport, and toxicity of Pb in the gastrointestinal tract.

5.11 LEAD-BINDING PROTEINS

Lead-binding proteins that are constitutively expressed within the cells and bind Pb can be classified into two types of protein. The first type of Pb-binding proteins are inducible, i.e., their concentration increases after exposure to Pb. The second type of Pb-binding proteins have binding sites that are saturable by Pb, but no discernible increase in protein content occurs after exposure to Pb. The second type is, perhaps, most pertinent to enzymes that can be inhibited by Pb.

The history of research on Pb-binding proteins dates back to 1936, when the presence of intranuclear inclusion bodies in the liver and kidney as manifestations of Pb poisoning was first described (Blackman, 1936). Later, detailed studies of the composition of renal tubular intranuclear Pb inclusion bodies and consequent alterations in mitochondrial structure and function followed.

5.11.1 Lead-Binding Proteins within Intranuclear Inclusion Bodies in Kidney

Goyer (1968) examined the renal tubules of rats fed 1% Pb acetate for up to 20 weeks, and found that dense, deeply staining intranuclear inclusions were located in the straight portion of the proximal tubules, accompanied by swollen, globular or ovoid, closely packed mitochondria with many marginated, irregular, or vesicular cristae. Accompanying these

mitochondrial changes was the presence of generalized aminoaciduria. Goyer et al. (1968) also isolated mitochondria from Pb-exposed and control rats and demonstrated that mitochondria from the Pb-exposed rats showed reduced rates of respiration and oxidative phosphorylation.

Lead within the kidneys in Pb-poisoned rats was found to be concentrated in the nuclei and, within nuclei, in the nuclear inclusion body (Goyer et al., 1970a,b). Choie and Richter (1972) showed that rapid induction of inclusion bodies by injections of Pb salts in the rat resulted in cytoplasmic inclusions, suggesting that they were precursors to the intranuclear inclusions. This was further confirmed by McLachlin et al. (1980) who showed in tissue culture studies of rat kidney cells incubated with lead that the cytoplasmic inclusion bodies preceded and disappeared shortly after the appearance of nuclear inclusion bodies.

Lead-containing nuclear inclusions were also found in organs other than the kidney, including liver and glial cells of the central nervous system (Goyer and Rhyne, 1973). Moore et al. (1973) dissolved the rat renal intranuclear inclusions in strong denaturing agents and found that the protein in the inclusions is acidic, with high levels of aspartic acid, glutamic acid, glycine, and cystine. Moore and Goyer (1974) later characterized the protein as a 27.5 kDa protein, which migrates as a single band on acrylamide gel electrophoresis. Repeated intraperitoneal injections of $CaNa_2EDTA$ resulted in the disappearance of the inclusion bodies in Pb-exposed rats, together with a marked decrease in kidney Pb levels (Goyer et al., 1978).

Shelton and co-workers have also explored the composition of Pb-binding proteins in the nuclear inclusion proteins of Pb-exposed rat kidneys. Shelton and Egle (1982) first described a 32 kDa protein with an isoelectric point of 6.3, which was isolated from the kidneys of rats treated with 1% Pb acetate in rat chow or 0.75% Pb acetate in drinking water for 13-17 weeks. In contrast to Goyer and co-workers, they used two-dimensional gel electrophoresis to isolate the protein from the nuclear inclusion bodies and demonstrated that it was present in Pb-exposed, but not control, kidneys (hence, inducible). This protein has been termed p32/6.3. Inhibitor studies with cycloheximide and actinomycin D (McLachlin et al., 1980; Choie et al., 1975) had indicated earlier that protein synthesis was required for induction of the nuclear and cytoplasmic inclusion bodies.

Egle and Shelton (1986) unexpectedly found that p32/6.3, now characterized by a monoclonal antibody, was constitutively present in the cerebral cortex, both in neurons and astrocytes. The protein was concentrated in the insoluble nuclear protein, findings similar to the

Pb-exposed kidney. Brain p32/6.3 was detected in rat, mouse, dog, man, and chicken. In rat brain, adult levels were achieved in 1 to 2 weeks after birth, whereas only trace amounts were found at 3 days. Brain p32/6.3 increased between postnatal days 10 to 12 in the guinea pig and days 15 to 21 in the rat, suggesting that the increase may be related in part to exposure to the external environment (Shelton et al., 1993). When neuroblastoma cells were cultured after 1-day and 3-day exposure to Pb, the abundance of p32/6.3 increased. Simultaneous incubation with Pb and cycloheximide or actinomycin D showed an increase in p32/6.3, suggesting that Pb selectively retards the degradation of the brain protein (Klann and Shelton, 1989). The amino acid composition of partially purified p32/6.3 revealed a high percentage of glycine, aspartic and glutamic acid (Shelton et al., 1990). Thus, the inducible protein, p32/6.3, can be extracted from nuclear inclusion bodies from the Pb-exposed rat kidney, and a similar or identical protein from adult rat brain. Whether the brain protein is constitutive or inducible by exposure to environmental Pb has yet to be determined. Selvin-Testa et al. (1991) and Harry et al. (1996) reported that developing rat brain astrocytes exposed to Pb developed an elevation in glial fibrillary acidic protein (GFAP), a developmentally-regulated protein. Harry et al. (1996) consider that the elevated levels of GFAP mRNA during the second postnatal week after Pb exposure may reflect the demand on astrocytes to sequester Pb.

Oskarsson and Fowler (1985) examined the influence of pretreatment with Pb by a single IP injection of Pb acetate (50 mg Pb per kg) 1, 3, and 6 days before injecting ^{203}Pb. Rats were sacrificed 24 h later and the kidneys were examined both microscopically and for the distribution of ^{203}Pb. At 3 days, rat kidneys displayed fibrillar cytoplasmic inclusions, but at 6 days, these inclusions were less prominent and intranuclear inclusions were observed. ^{203}Pb uptake at 6 days was maximal in the purified nuclear fraction and in the nuclear inclusion bodies (7 $\times$ and 20 $\times$ control, respectively).

5.11.2 Cytoplasmic Lead-binding Proteins in Kidney and Brain

The remaining studies of non-Pb-stimulated cytoplasmic kidney and brain Pb-binding proteins have been provided by Fowler and associates.

The first study (Oskarsson et al., 1982) reported on the Pb-binding proteins in kidney postmitochondrial cytosolic fractions. Binding of ^{203}Pb was found in two protein fractions of control kidneys with molecular weights of 11.5 and 63 kDa. Binding was markedly decreased

after Pb pretreatment. The use of cadmium to stimulate metallothionein synthesis did not increase ^{203}Pb binding to the 11.5 kDa protein. The two binding proteins were also present in brain, but not in liver or lung. Subsequently, Mistry et al. (1985) demonstrated three Pb-binding proteins (11.5, 63, and >200 kDa) in rat kidney cytosol, which had binding characteristics of high affinity, low capacity with respective K_d values of 13, 40, and 123 nM. The 11.5 kDa and, possibly, the 63 kDa proteins were capable of translocating Pb into the nucleus as shown by uptake of ^{203}Pb into nuclei incubated with tagged cytosolic proteins. Goering and Fowler (1984) showed that the 11.5 kDa protein, but not the 63 kDa protein was capable of reversing Pb-induced ALAD inhibition in liver homogenates. This effect was mediated both by chelation of Pb by the Pb-binding protein and by donation of zinc to ALAD (Goering and Fowler, 1985). Various divalent metal ions influence the binding of Pb to the rat kidney cytosolic binding proteins, with an order of displacement of $Cd^{2+}>Zn^{2+}>Pb^{2+}$. Ca^{2+} had no effect, while Fe^{2+} had a cooperative effect (Mistry et al., 1986). These observations may account for the previously demonstrated effect of concomitant Pb and cadmium administration in reducing total kidney Pb (Mahaffey et al., 1981) and preventing the development of intranuclear inclusion bodies (Mahaffey and Fowler, 1977).

Later studies by Fowler and Duval (1991) identified the rat renal Pb-binding protein as a cleavage product of α2-microglobulin, with a K_d of 10^{-8} M Pb. There are two forms of the protein in the kidney, differentiated by the cleavage of the first 9-N terminal residues from the higher-molecular weight form. Other studies by Smith et al. (1998) found two Pb-binding proteins in environmentally exposed human kidneys, identified as acyl-CoA binding protein (ACBP) or diazepam binding inhibitor (molecular weight 9 kDa) and thymosin β4 (molecular weight 5 kDa). These polypeptides have a high affinity for Pb (K_d~14 nM).

In rat brain, Goering et al. (1986) and DuVal and Fowler (1989) explored the effects of environmental Pb on Pb-binding proteins and the ability of rat brain Pb-binding proteins to diminish the inhibition of hepatic ALAD by Pb (liver does not contain the Pb-binding protein). In the first study, a brain protein of 12 kDa was described, in comparison to the kidney Pb-binding protein of 9 kDa. Both competition of Pb binding between the brain Pb-binding protein and ALAD and donation of zinc by the brain protein (shown by ^{65}Zn uptake) were found to account for the decreased ALAD inhibition. In the second study the rat brain Pb-binding protein was described as having a molecular weight of 23 kDa, with significant levels of

glutamic acid, aspartic acid, and cysteine. Polyclonal antibody to rat renal Pb-binding proteins showed a lack of reactivity with the brain protein, indicating that the proteins are immunologically distinct.

Fowler et al. (1993) examined monkey kidney and brain from non-Pb-treated animals and isolated Pb-binding proteins that also had a relatively high content of aspartic and glutamic amino acid residues and were similar in size to the rat Pb-binding proteins. Polyclonal antibodies to α-2 microglobulin and metallothionein did not cross-react with either monkey kidney or brain proteins. Quintanilla-Vega et al. (1995) isolated a thymosin β4 and a second, as yet unidentified, protein with a molecular weight of 20 kDa and a pI of 5.9 from brains of environmentally Pb-exposed humans.

Lead also has been reported to bind to p32/6.3, a low abundance, highly conserved nuclear matrix protein that becomes a prominent component of Pb-induced intranuclear inclusion bodies (Klann and Shelton, 1989). Expression of this protein increases significantly during ontogeny, and was proposed to be useful as an indicator of neuronal maturation (Klann and Shelton, 1990). Expression also increases markedly in the presence of acute Pb^{2+} exposure in vitro, suggesting that Pb^{2+} either structurally alters the protein or inhibits a protease for which p32/6.3 is a substrate (Shelton *et al.*, 1993).

Recently an astroglial glucose-regulated protein (GRP78) has been identified that acts as a molecular chaperone in endoplasmic reticulum (Qian *et al.*, 2000, 2005a). Intracellular levels of this protein are increased in cultured astroglia during a 1- week exposure to Pb^{2+}. GRP78 depletion significantly increased the sensitivity of cultured glioma cells to Pb^{2+}, as indicated by the generation of reactive oxygen species. This suggests that GRP78 is a component of the intracellular tolerance mechanism that handles high intracellular Pb accumulation through a direct interaction. Thus, it appears that Pb^{2+} directly targets the protein and induces its compartmentalized redistribution, enabling it to play a protective role in Pb neurotoxicity. The generation of reactive oxygen species also has been reported to occur via Pb^{2+} binding to astroglial copper-transporting ATPase, resulting in disruption of copper homeostasis (Qian *et al.*, 2005b).

5.11.3 Lead-binding Proteins in Erythrocytes

Intra-erythrocytic Pb binding was initially attributed primarily to hemoglobin, molecular weight 64 kDa (Barltrop and Smith, 1972; Raghavan and Gonick, 1977; Ong and Lee, 1980; Lolin and O'Gorman, 1988), but more recent studies have ascribed the major Pb binding to ALAD, molecular weight 240–280 kDa. In contrast to this protein, several studies have focused on an inducible low molecular weight protein in workers chronically exposed to Pb and which seems to have a protective effect. The first recognition of this protein was by Raghavan and Gonick (1977) who found an ~10 kDa protein in Pb workers but not in controls, following Sephadex G-75 fractionation (Figure 5-22). Upon subsequent SDS-polyacrylamide gel electrophoresis, the protein split into two bands, only the uppermost of which contained Pb (Figure 5-23).

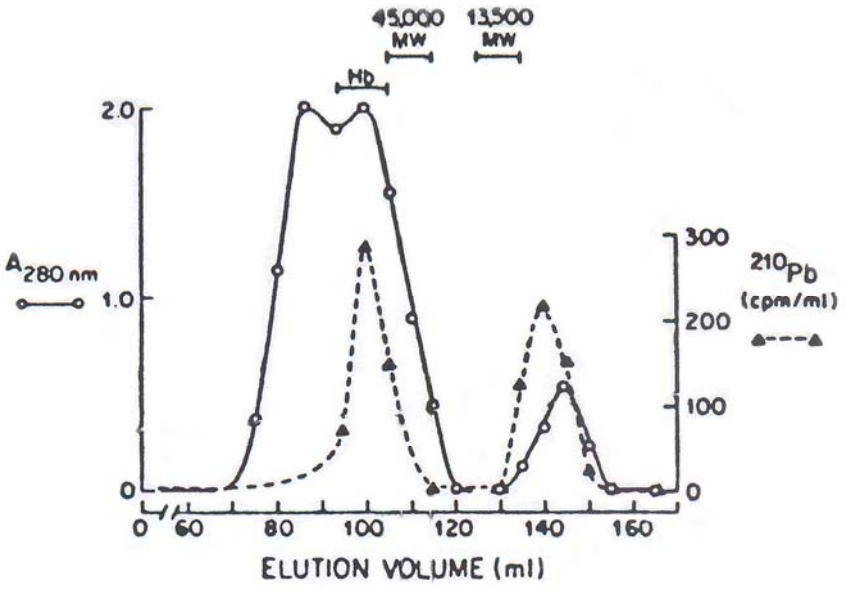

Figure 5-22. Sephadex G-75 gel filtration of RBC hemolysate from lead-exposed individual. Ultraviolet absorption and radioactivity of ^{210}Pb are plotted against elution volume. The column was calibrated with ovalbumin (mol wt 45,000) and ribonuclease (mol wt 13,700). Also indicated is the locus of hemoglobin (Hb). Hemolysates from normal control individuals showed no UV absorption or radioactivity in the volume eluting between 130 and 155 mL.

Source: Raghavan and Gonick (1977) with permission.

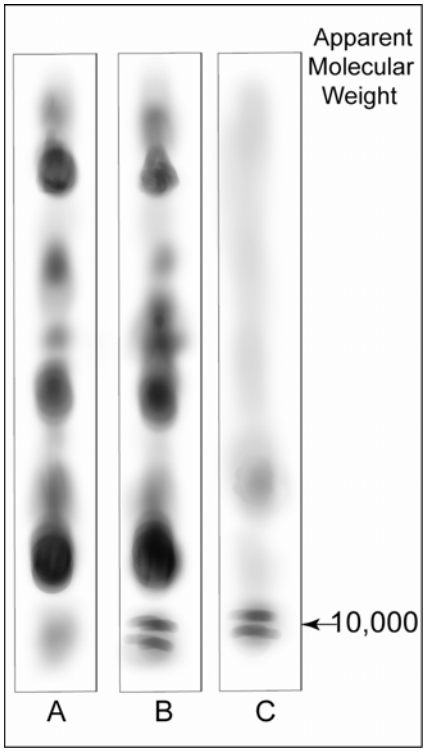

Figure 5-23. SDS-polyacrylamide gel electrophoresis of RBC hemolysates from normal control (A) and lead-exposed individuals (B), and of low-mol-wt. lead-binding protein (C) stained with coomassie blue.

Source: Raghavan and Gonick (1977) with permission.

Raghavan et al. (1980) then went on to fractionate the erythrocyte Pb into a hemoglobin fraction, a 10 kDa fraction, free Pb, and a "residual Pb" fraction thought to be composed of membrane Pb and a high-molecular weight fraction. Lead workers manifesting toxicity at both high blood Pb and relatively low blood Pb levels showed high levels of residual Pb, attributed in the workers with toxicity at low blood leads to a very low quantity of the 10 kDa fraction. In a follow-up study, Raghavan et al. (1981) reported elevated levels of Pb in the high molecular weight fraction (pre-hemoglobin) and in the membrane fraction in workers with toxicity at both high and low blood Pb levels. Again, those with toxicity at low blood Pb had low levels of the Pb bound to the 10 kDa protein. Membrane Pb was found to correlate inversely with membrane NaK-ATPase; no correlation was seen with total blood Pb.

Gonick et al. (1985) partially purified the 10 kDa protein by HPLC using a protein I-125 column followed by isoelectric focusing on a sucrose gradient column. Three protein peaks resulted: one of 30 kDa, and two of 10 kDa. Only one of the latter peaks contained Pb. This peak had a pI of 5.3 and a molecular weight, determined by SDS-PAGE, of 12 kDa. The majority of Pb was found in this peak, which also contained calcium, zinc, and cadmium. Amino acid analysis showed a very high percentage of glycine (44%) and lower quantities of histidine, aspartic acid, and leucine.

Ong and Lee (1980) studied the distribution of ^{203}Pb in components of normal human blood. Ninety-four percent of ^{203}Pb was incorporated into the erythrocyte and 6% remained inthe plasma. SDS-PAGE of plasma showed that 90% was present in the albumin fraction. Within the erythrocyte membrane, the most important binding site was the high molecular weight fraction, about 130–230 kDa. Within the erythrocytic cytoplasm, the protein band associated with ^{203}Pb had a molecular weight of 67 kDa as shown by the elution characteristics on G-75 chromatography. This was thought to be hemoglobin.

Lolin and O'Gorman (1988) and Church et al. (1993 a,b), following the same procedure as Raghavan and Gonick (1977), confirmed the findings of a low molecular weight protein in the erythrocytes of Pb workers, but not found in control patients. Lolin and O'Gorman (1988) quantitated the protein, which ranged from 8.2 to 52.2 mg/L RBC in Pb workers, but found none in controls, again implying it to be an inducible protein. They found that the low molecular weight protein first appeared when the blood Pb concentration exceeded 39 µg/dL. A positive correlation was seen between the amount of the intra-erythocytic low molecular weight protein and dithiothreitol-activated ALAD activity but not the non-activated activity. Church et al. (1993a,b) also confirmed the findings of Raghavan and Gonick (1977). In 1993a, they described two patients with high blood Pb levels: an asymptomatic worker with a blood Pb of 180 µg/dL, and a symptomatic worker with a blood Pb of 161 µg/dL. In the first patient, ~67% of the erythrocyte Pb was bound to a low molecular weight protein of ~6–7 kDa. In the second patient, the protein only contained 22% of the total erythrocytic Pb. Church et al. (1993b) found that a sample of the low molecular weight protein purified from Pb workers, which they termed protein M, had characteristics of metallothionein, such as a molecular weight of 6.5 kDa, a pI between 4.7 and 4.9, and a greater UV absorbance at 254 nm than at 280 nm. Amino acid composition showed 33% cysteine but no aromatic amino acids. This composition differed from that of the

low molecular weight protein described by Gonick et al. (1985), which had a molecule weight of 12 kDa, a pI of 5.3, and amino acid analysis that showed no cysteine. This discrepancy might be explained by a combined Pb and cadmium exposure in the Church et al. (1993b) study, which may have produced a Pb-thionein.

Xie et al. (1998) used a Biogel A column instead of Sephadex G-75 to separate Pb-binding proteins from erythrocyte hemolysates from a control patient and from Pb-exposed workers. They clearly showed that the major Pb-binding was associated with a large molecular weight protein, consistent with ALAD, in both the controls and Pb workers. When they added increasing amounts of Pb to the blood of the control patient, a second low molecular weight protein peak occurred, in which Pb binding was larger than the ALAD peak (Figure 5-24). This second peak was also seen in a chronically Pb-exposed worker (Figure 5-25) and was estimated to be less than 30 kDa in molecular weight. Thus these results are consistent with the aforementioned studies.

5.11.4 Lead-binding Proteins in Rat Liver

Sabbioni and Marafante (1976) explored the distribution of ^{203}Pb in rat whole tissue as well as in subcellular liver fractions. By far the largest quantity of Pb recovered was in the kidney, with lesser amounts in liver, spleen, and blood. Upon subcellular fractionation of the liver, the majority of ^{203}Pb was found in the nuclei, and most of the Pb was detected in the nuclear membrane fraction, bound exclusively to membrane proteins. The intranuclear Pb was associated with histone fractions. As reported by Oskarsson et al. (1982), Pb binding proteins were not found in the cytoplasm of the liver.

5.11.5 Lead-binding Proteins in Intestine

Fullmer et al. (1985) showed in the chick and cow that, although Pb does not directly stimulate Pb-binding proteins in the intestine, Pb can displace calcium from calcium-binding proteins; and, thus, calcium-binding proteins may play a role in intestinal Pb transport. Purified calcium-binding protein from chick and cow, as well as calmodulin, troponin C, and oncomodulin were dialyzed against added labeled and unlabeled Pb or calcium. Results disclosed high affinity binding sites, with greater affinity for Pb than for calcium. Similar results

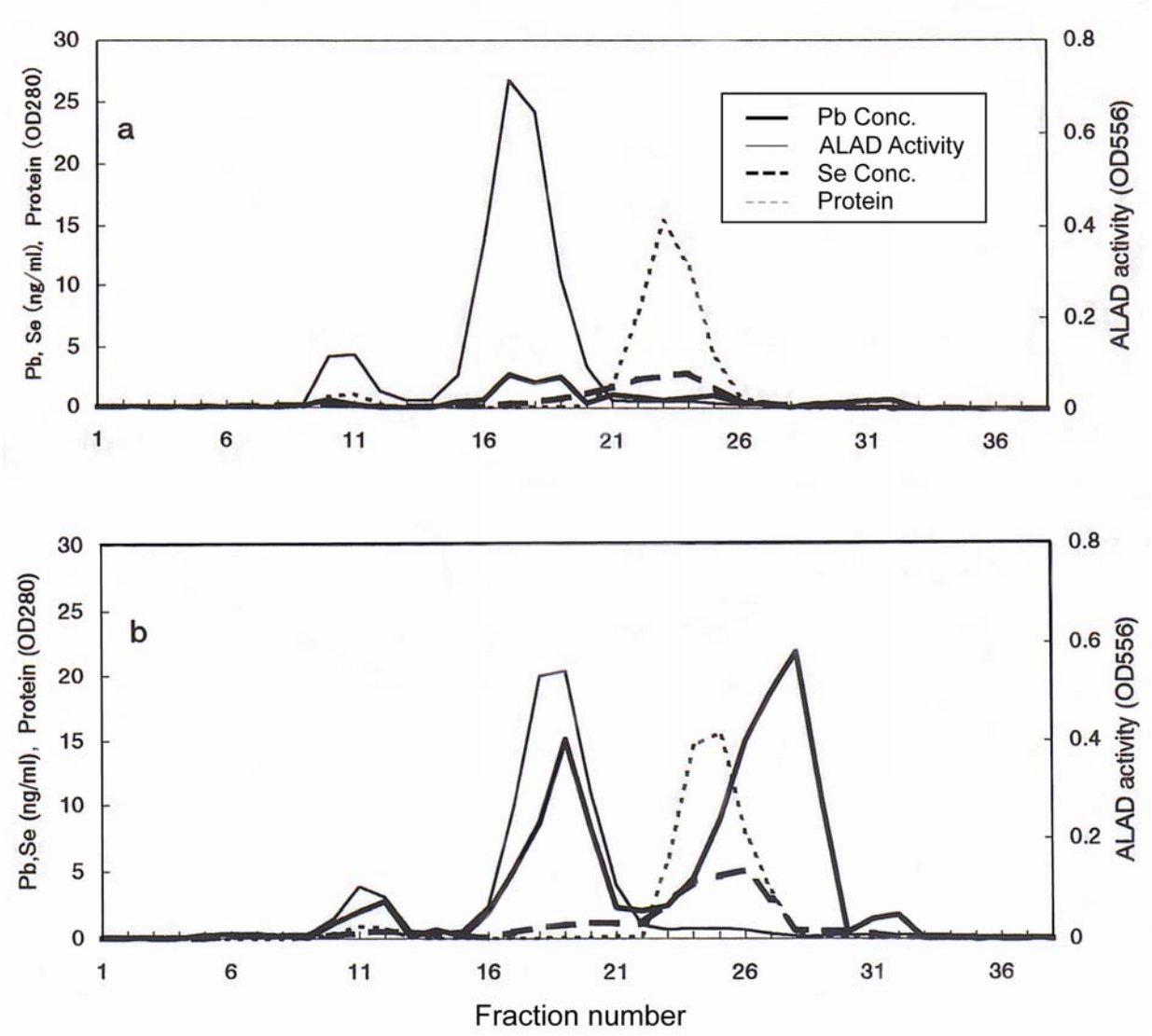

Figure 5-24. Chromatographic profiles of protein, ALAD activity and lead in human erythrocytes incubated with 5% glucose solution containing lead acetate. Blood was incubated (a) without lead (b) 10 µM lead (final concentrations).

Source: Adapted from Xie et al. (1998).

were obtained with calmodulin, troponin C, and oncomodulin, all members of the troponin C superfamily of calcium-binding proteins.

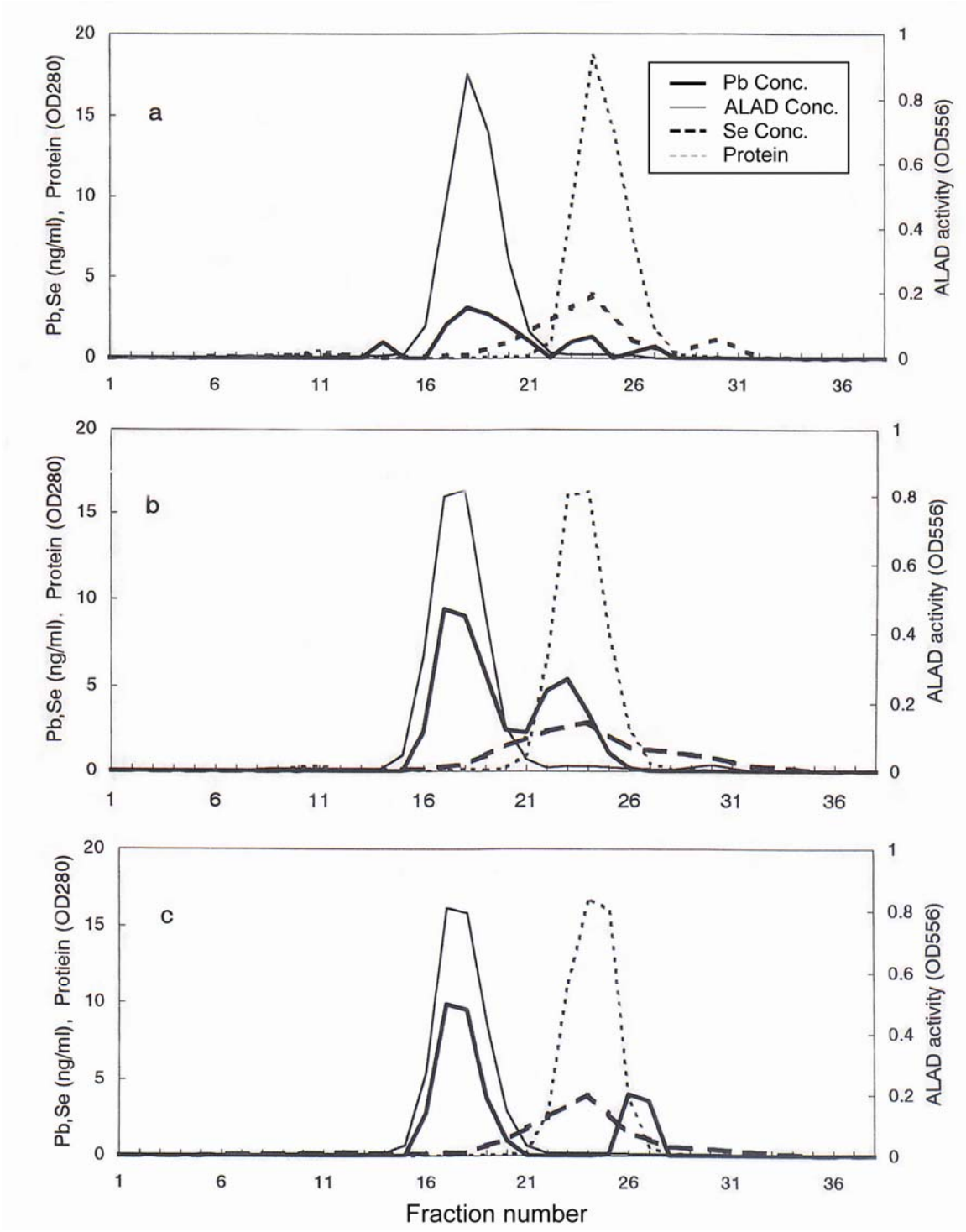

Figure 5-25. Chromatic profiles of protein, ALAD activity, lead, and Se in the erythrocytes of lead-exposed workers. (a) control, (b) subacute exposure, (c) chronic exposure.

Source: Xie et al. (1998) with permission.

5.11.6 Lead-binding Protein in Lung

Singh et al. (1999) described intracellular Pb-inclusion bodies in normal human lung small airway epithelial cells cultured with either Pb chromate particles or sodium chromate. Cells exposed to both forms of chromate underwent dose-dependent apoptosis. Lead-inclusion bodies were found in nucleus and cytoplasm of Pb chromate, but not sodiumchromate, treated cells. Lead, but not chromium, was detected in the inclusion bodies by energy-dispersive X-ray analysis. The protein within the inclusion bodies has not been analyzed.

5.11.7 Relationship of Lead-binding Protein to Metallothionein

Similarities of Pb-binding protein to metallothionein have been discussed earlier. Maitani et al. (1986) commented that hepatic zinc-metallothionein could be induced by intravenous and intraperitoneal injections of Pb into mice, but not by subcutaneous injection. Ikebuchi et al. (1986) found that a sublethal dose of Pb acetate injected intraperitoneally into rats induced the synthesis of a Pb-metallothionein in addition to zinc-metallothionein. The Pb-metallothionein contained 28% half-cysteine and cross-reacted with an antibody against rat zinc-thionein II.

Goering and Fowler (1987 a,b) demonstrated that pretreatment of rats with zinc 48 and 24 h prior to injection of ^{203}Pb resulted in both zinc and Pb co-eluting with a zinc-thionein fraction on Sephadex G-75 filtration. In addition, both purified zinc-thionein-I and II bound ^{203}Pb in vitro. Gel filtration of incubates containing liver ALAD and ^{203}Pb demonstrated that the presence of zinc-thionein alters the cytosolic binding pattern of Pb, with less binding to ALAD. Zinc-thionein also donates zinc to activate ALAD. Goering and Fowler (1987b) found that pretreatment of rats with either cadmium or zinc affected liver ALAD activity when incubated with Pb. Liver and kidney zinc-thioneins, and to a lesser extent, cadmium, zinc-thionein decreased the free pool of Pb available to interact with ALAD, resulting in attenuated ALAD inhibition. Liu et al. (1991) further showed that zinc-induced metallothionein in primary hepatocyte cultures protects against Pb-induced cytotoxicity, as assessed by enzyme leakage and loss of intracellular potassium.

Qu et al. (2002) and Waalkes et al. (2004) have shown that metallothionein-null phenotypic mice are more susceptible to Pb injury over a 20-week period than wild type mice. Unlike the wild type mice, Pb-treated metallothionein-null mice showed nephromegaly and significantly decreased renal function after exposure to Pb. The metallothionein-null mice

accumulated less renal Pb than wild type and formed no Pb-inclusion bodies. When the observations were extended to 104 weeks, renal proliferative lesions (adenoma and cystic tubular atypical hyperplasia) were more common and severe in metallothionein-null than in wild type mice. A metastatic renal cell carcinoma occurred in a metallothionein-null mouse, whereas none occurred in wild type mice. Such studies lend credence to the view that metallothinein, or a closely related gene, is involved in the formation of Pb-binding proteins in the kidney.

5.11.8 Is ALAD an Inducible Enzyme and Is It the Principal Lead-binding Protein in the Erythrocyte?

The enzyme ALAD has been found to be the most sensitive indicator of Pb exposure and toxicity (Granick et al., 1973, Buchet et al., 1976). In the 1980s, two articles were presented appearing to show that ALAD is inducible after Pb exposure in humans. By comparing a nonexposed control population of Pb workers and assaying ALAD by means of immunoassay or as "restored" ALAD activity (i.e., incubation with heat, zinc and dithiothreitol) both articles indicated that the amount of ALAD, as contrasted to ALAD activity, was increased by Pb exposure (Fujita et al., 1982; Boudene et al., 1984). Similar findings were reported for the rat (Fujita et al., 1981). Subsequent studies have focused on the effect of ALAD polymorphism on the susceptibility to Pb intoxication. ALAD is a zinc-containing enzyme, which catalyzes the second step of heme synthesis, i.e., catalyzes the condensation of two delta-aminolevulinic acid molecules into one molecule of porphobilinogen (Boudene et al., 1984). It is a polymorphic protein with three isoforms: ALAD-1, ALAD 1-2, and ALAD 2-2. Several studies have shown that, with the same exposure to Pb, individuals with the ALAD-2 gene have higher blood Pb levels (Astrin et al., 1987; Wetmur, 1994; Wetmur et al., 1991; Smith et al., 1995a; Bergdahl et al., 1997; Perez-Bravo et al., 2004; Kim et al., 2004). Initially, it was thought that these individuals might be more susceptible to Pb poisoning (Wetmur et al., 1991), but it is now appreciated that the ALAD-2 gene offers protection against Pb poisoning by binding Pb more securely (Kelada et al., 2001). In support of this statement, it can be cited that individuals with the ALAD 1-2/2-2 genotypes, in comparison to those with the ALAD 1-1 genotype, have not only higher blood Pb but also decreased plasma levulinic acid (Schwartz et al., 1997a), lower zinc protopophyrin (Kim et al., 2004), lower cortical bone Pb (Smith et al., 1995b), and lower amounts of DMSA-chelatable Pb (Schwartz et al., 1997b, 2000).

The significance of erythrocyte ALAD binding to Pb was initially confirmed by a study by Bergdahl et al. (1997), in which an FPLC Superdex 200 HR 10/30 chromatographic column coupled to ICP-MS (for determination of Pb) was used to examine erythrocytes from Pb workers and controls. They found the principal Pb-binding protein peak to be 240 kDa, rather than the presumed hemoglobin peak reported by Barltrop and Smith (1972) and Raghavan and Gonick (1977), using Sephadex G-75 chromatography. This was shown to be ALAD by binding to specific ALAD antibodies. Two additional smaller Pb-binding peaks of 45 kDA and 10 kDa were also seen, but not identified. Bergdahl et al. (1997) attributed the discrepancies in the studies to the fact that Sephadex G-75 separates proteins in the range of 3 to 80 kDa, making the separation of hemoglobin (molecular weight 64 kDa) from ALAD (molecular weight 240–280 kDa) very difficult. In addition, the earlier studies had utilized binding of ^{203}Pb or ^{210}Pb to identify the binding proteins, a technique which may have skewed the findings if ALAD were already saturated. ALAD binding capacity for Pb has been measured at 85 µg/dL in erythrocytes or 40 µg/dL in whole blood (Bergdahl et al., 1998), which would permit a greater degree of binding to the low molecular weight component when blood Pb exceeded 40 µg/dL. Bergdahl et al. (1998) have speculated that the low molecular weight component might be acyl-CoA-binding protein, identical to the kidney Pb-binding protein described by Smith et al. (1995b). Goering and Fowler (1987) had reported earlier that the presence of low molecular weight high affinity (K_d 10^{-8}M) Pb-binding proteins in kidney and brain served as protection against ALAD inhibition in those organs, whereas the absence of the low molecular weight proteins in liver contributed to the greater sensitivity to ALAD inhibition in that organ.

A summary of key findings on Pb-binding protein is presented below, whereas more detailed summarization of pertinent individual studies is provided in Table AX5-11.1.

5.11.9 Summary

- Nuclear inclusion bodies stimulated by Pb have been extensively investigated. The nuclear inclusion body within the kidney and brain of rats contains a relatively insoluble protein, tentatively identified as a 32 kDa protein with an isoelectric point of 6.3. The nuclear inclusion body is preceded by the development and subsequent disappearance of a cytoplasmic inclusion body. Whether the proteins within these two inclusion bodies are similar or the same remains to be determined.

- There appears to be a consensus that the enzyme, ALAD (a 280 kDa protein), is inducible and is the major Pb-binding protein within the erythrocyte. ALAD polymorphism influences the degree of Pb-binding, as the ALAD-2 phenotype binds more Pb in a nontoxic fashion than ALAD-1. What is more confusing is the nature and importance of the low molecular weight erythrocytic Pb-binding protein. There is no doubt that it appears in Pb-exposed workers but not in controls and that its molecular weight is ~10 kDa. The in vitro addition of Pb to erythrocytes of controls results in progressively increasing Pb binding to a low molecular weight protein peak migrating in the same position as the low molecular weight protein from Pb workers. This confirms the fact that once the binding capacity of ALAD is saturated, Pb shifts to the low molecular weight protein. The nature of the low-molecular weight protein is also questionable; it has been variously identified as a 12 kDa protein with a high percentage of glycine plus histidine, aspartic acid, and leucine and as a 6.5 kDa molecule with a large percentage of cysteine and a greater UV absorbance at 254 than 280 nm. The latter findings suggest that the protein might be a metallothionein.

- Metallothionein is a protein that is mildly inducible by Pb but to a much greater degree by zinc and cadmium. What is more significant is that Pb binds to pre-formed metallothionein, stimulated by zinc or cadmium, so that under these conditions a Pb-thionein forms. Thus, if concomitant Pb and cadmium exposure occurred in Pb workers that could account for the finding of a metallothionein-like protein in those workers.

- The possible role of metallothionein as a renal Pb-binding protein assumes greater importance because of the work showing that metallothionein-null mice failed to respond to Pb exposure by developing intranuclear Pb inclusion bodies or greatly increased Pb content of the kidneys.

- Extensive studies of cytoplasmic Pb-binding proteins in non-Pb-treated rats, human, and monkeys have been reported. The Pb-binding protein in rat kidney has been identified as a cleavage product of α-2 microglobulin. The low molecular weight Pb-binding proteins in human kidney have been identified as thymosin β4 (molecular weight 5 kDa) and acyl-CoA binding protein (molecular weight 9 kDa). In human brain, the Pb-binding proteins were thymosin β4 and an unidentified protein of 23 kDa. Antibodies to α-2 microglobulin and metallothionein did not cross-react with monkey kidney or brain Pb-binding proteins, suggesting species differences. Whether the low molecular weight human kidney and brain Pb-binding proteins are similar or identical to the low molecular weight Pb-binding proteins in erythrocytes is at present unknown. Perhaps some clarification would be provided were subsequent investigators to contrast normal with Pb-exposed rats and to measure the resting and inducible Pb-binding protein levels in kidney, brain, and erythrocyte.

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6. EPIDEMIOLOGIC STUDIES OF HUMAN HEALTH EFFECTS ASSOCIATED WITH LEAD EXPOSURE

6.1 INTRODUCTION

This chapter assesses epidemiologic information regarding the biological effects of lead (Pb) exposure, with emphasis on (1) qualitative characterization of Pb-induced effects and (2) delineation of concentration-response relationships for key health effects of most concern. Epidemiologic studies linking Pb exposure to health effects were earlier assessed in the 1986 Air Quality Criteria for Lead (U.S. Environmental Protection Agency, 1986a), an associated 1986 Addendum (U.S. Environmental Protection Agency, 1986b), and a 1990 Supplement (U.S. Environmental Protection Agency, 1990). Environmental exposures to Pb result from human contact with multimedia exposure pathways (e.g., air, food, water, surface dust), as discussed extensively in Chapters 3 and 4 of this document. In this chapter, while recognizing the multimedia nature of Pb exposure of the general population, Pb exposure is generally indexed by tissue Pb concentrations measured in biomarkers such as blood and bone. Many earlier studies reported Pb effects on child development (psychometric intelligence), blood pressure and related cardiovascular endpoints, heme biosynthesis, kidney, and reproduction and development. Numerous more recent epidemiologic studies discussed in this chapter have further evaluated these relationships to Pb exposure, thereby providing an expanded basis for assessment of health effects associated with exposure to Pb at concentrations currently encountered by the general U.S. population.

Special emphasis is placed here on discussion of the effects of Pb exposure in children. Children are particularly at risk due to sources of exposure, mode of entry, rate of absorption and retention, and partitioning of Pb in soft and hard tissues. The greater sensitivity of children to Pb toxicity, their inability to recognize symptoms, and their dependence on parents and healthcare professionals make them an especially vulnerable population requiring special consideration in developing criteria and standards for Pb.

As discussed elsewhere in this document (Chapter 5), extensive experimental evidence also links Pb exposure with health effects in laboratory animals. Thus, many of the reported epidemiologic associations of Pb health effects have considerable biological credibility.

Accordingly, the new epidemiologic studies of Pb assessed here are best considered in combination with information from the other chapters on Pb exposure and on toxicological effects of Pb in animals. The epidemiologic studies constitute important information on associations between health effects and exposures of human populations to "real world" Pb concentrations and also help to identify susceptible subgroups and associated risk factors.

6.1.1 Approach to Identifying Lead Epidemiologic Studies

Numerous Pb epidemiologic papers have been published since completion of the 1986 Lead AQCD/Addendum, and 1990 Supplement. A systematic approach has been employed to identify relevant new epidemiologic studies for consideration in this chapter. In general, an ongoing literature search has been used in conjunction with other strategies to identify Pb epidemiologic literature pertinent to developing criteria for the National Ambient Air Quality Standards (NAAQS) for Pb. A publication base was established using Medline, Pascal, BIOSIS, and Embase, and a set of search terms aimed at identifying pertinent literature.

While the above search regime accessed much of the pertinent literature, additional approaches augmented such traditional search methods. For example, a Federal Register Notice was issued requesting information and published papers from the public at large. Also, non-EPA chapter authors, expert in this field, identified literature on their own; and EPA staff also identified publications as part of their assessment and interpretation of the literature. Lastly, additional potentially relevant publications have been identified and included as a result of external review of this draft document by the public and CASAC. The principal criteria used for selecting literature for the present assessment is to focus mainly on those identified studies that evaluate relationships between health outcome and Pb exposure at concentrations in the range of those currently encountered in the United States. New studies published or accepted for publication through December 2005, as identified using the approaches above, have generally been included in this Lead Air Quality Criteria Document (Lead AQCD), and additional efforts have been made to identify and assess a few more recent but important studies.

6.1.2 Approach to Assessing Epidemiologic Evidence

Epidemiologic studies have evaluated Pb effects on a wide range of health endpoints that include, but are not limited to: neurotoxic effects (e.g., psychometric intelligence, behavioral

disturbances, and neurodevelopmental deficits), renal effects, cardiovascular effects, reproductive and developmental effects, genotoxic and carcinogenic effects, and immune effects. The epidemiologic strategies most commonly used in Pb health studies are: (1) cross-sectional studies that examine the exposure and health outcome at a single point in time; and/or (2) prospective longitudinal cohort studies that follow a group of individuals over time. Both of these are types of observational, rather than experimental, studies.

An overall approach useful for assessing epidemiologic evidence was stated in the 2004 PM AQCD (U.S. Environmental Protection Agency, 2004) and is summarized here. That is, the critical assessment of epidemiologic evidence presented in this chapter is conceptually based upon consideration of salient aspects of the evidence of associations so as to reach fundamental judgments as to the likely causal significance of the observed associations (see Hill, 1965). The general evaluation of the strength of the epidemiologic evidence reflects consideration not only of the magnitude and precision of reported Pb effect estimates and their statistical significance, but also of the robustness of the effects associations. Statistical significance corresponds to the allowable rate of error (Type I error) in the decision framework constructed from assuming that a simple null hypothesis of no association is true. It is a conditional probability; for statistical significance, typically there is a less than 0.05 chance of rejecting the null hypothesis given that it is true. Robustness of the associations is defined as stability in the effect estimates after considering a number of factors, including alternative models and model specifications, potential confounding by copollutants, as well as issues related to the consequences of measurement error.

Consideration of the consistency of the effects associations, as discussed in the following sections, involves looking across the results obtained by various investigators in different populations, locations, and times. Relevant factors are known to exhibit much variation across studies, e.g., (1) presence and levels of other toxicants or pollutants of concern and (2) relevant demographic factors related to sensitive subpopulations. Thus, consideration of consistency is appropriately understood as an evaluation of the similarity or general concordance of results, rather than an expectation of finding quantitative results within a very narrow range.

Looking beyond the epidemiologic evidence, evaluation of the biological plausibility of the Pb-health effects associations observed in epidemiologic studies reflects consideration of both exposure-related factors and dosimetric/toxicologic evidence relevant to identification of

potential biological mechanisms underlying the various health outcomes. These broader aspects of the assessment are only touched upon in this chapter but are more fully addressed and integrated in Chapter 8 (Integrative Synthesis) discussions.

In assessing the relative scientific quality of epidemiologic studies reviewed here and to assist in interpreting their findings, the following considerations were taken into account:

(1) To what extent are the biological markers used of adequate quality and sufficiently representative to serve as credible exposure indicators, well-reflecting interpersonal differences in exposure for specified averaging times?

(2) Were the study populations well defined and adequately selected so as to allow for meaningful comparisons between study groups or meaningful temporal analyses of health effects results?

(3) Were the health endpoint measurements meaningful and reliable, including clear definition of diagnostic criteria utilized and consistency in obtaining dependent variable measurements?

(4) Were the statistical analyses used appropriate, as well as being properly performed and interpreted?

(5) Were likely important covariates (e.g., potential confounders or effect modifiers) adequately controlled for or taken into account in the study design and statistical analyses?

(6) Were the reported findings internally consistent, biologically plausible, and coherent in terms of consistency with other known facts?

These guidelines provide benchmarks for judging the relative quality of various studies and in assessing the overall body of epidemiologic evidence. Detailed critical analysis of all epidemiologic studies on Pb health effects, especially in relation to all of the above questions, is beyond the scope of this document.

6.1.3 Considerations in the Interpretation of Epidemiologic Studies of Lead Health Effects

Prior to assessing results from recent Pb epidemiologic studies, issues and questions arising from study designs and analysis methods used in the evaluation of Pb health effects are first briefly discussed here. Study design can restrict the health effect parameters that can be estimated. Separate considerations need to be made for acute versus chronic effect studies, as well as individual versus aggregate-level analyses. Issues include measurement error, the

functional form of relationships (especially at low Pb exposure levels) and the potential for confounding. Aspects of these issues are briefly noted below and are then considered as studies are reviewed in the following sections on specific health effect endpoints. These epidemiologic considerations are further examined in Section 6.10 and Chapter 8 (Integrative Synthesis).

Measurement error is an important factor to consider, both for measurement of the health effect outcome and the representativeness of the biomarkers of exposure (principally blood and bone Pb) used in most key epidemiologic studies. For health outcome measures, the reliability and validity of the measurements need to be assessed. In addition, the appropriateness of the outcome measure for studying the hypothesis of interest needs to be determined. The critical issues of outcome measurement and classification are, to some extent, endpoint-specific and are therefore discussed further in ensuing individual sections.

Exposure misclassification can result in a notable reduction of statistical power in studies, especially in those that focus on the lower end of the exposure range. Limitations of blood Pb as an exposure index include use of a single blood Pb concentration to represent Pb body burden. Also of concern is the most relevant blood sample collection time point in evaluating possible associations with health outcomes (e.g., blood Pb values at ~2 years of age when peak Pb exposure is expected versus those at later time point(s) concurrent with measurement of effect). Another consideration is that similar blood Pb levels in two individuals do not necessarily reflect similar body burdens. An added complication is that the relationship between Pb intake and blood Pb concentration is curvilinear. Bone Pb determinations are typically considered a measure of longer-term Pb exposure; but, the X-ray fluorescence (XRF) method typically used to assess Pb levels in bone also has limitations, including the relatively high minimum detection limit. The type of bone measured to determine Pb exposure is another important aspect.

The relationship between a measurement of a health outcome endpoint and an estimate of Pb exposure based on a biomarker is an important concept. Modeling this relationship provides a numerical slope that quantifies the relationship between Pb exposure and health outcome. These models must address differences in the relationship at different concentration ranges of exposure and present the functional form that best describes such data. Various models, both linear and nonlinear, have been considered to examine Pb exposure-health effect relationships. This is especially important at low Pb exposures. For example, a curvilinear relationship has been reported for neurodevelopmental and cardiovascular outcomes at low Pb exposure levels.

Depending on the subjects being examined for Pb exposure effects, various other factors can lead to confounding of the relationship being considered. Potential confounding factors largely depend on the health outcome of interest and the study population. Some potential confounding factors in children, for whom the major health concerns include neurological and developmental deficiencies, include: socioeconomic status (SES); nutritional status; quality of home environment (e.g., HOME score); parental education; parental IQ; and birth weight, as a few examples. For adults, factors that may confound the association between Pb exposure and cardiovascular health outcomes include: age; diet; alcohol use; smoking; and potential for copollutant exposures, such as to cadmium (Cd). For adult neurotoxic effects, potential confounders include age, education, depressive symptoms, medications, alcohol use and smoking. Control for potential confounding factors can be attempted at the study design phase and/or during statistical analysis.

6.1.4 Approach to Presenting Lead Epidemiologic Evidence

In the main body of this chapter, each section starts by concisely highlighting important points derived from the 1986 Lead AQCD/Addendum and the 1990 Supplement. Particular emphasis is focused on studies and analyses that provide pertinent information of importance for the critical assessment of health risks from Pb exposure. Not all studies are accorded equal weight in the overall interpretive assessment of evidence regarding Pb-associated health effects. Among well-conducted studies with adequate control for confounding, increasing scientific weight is accorded in proportion to the precision of their effect estimates. To ensure a thorough appraisal of the evidence, more detailed information on key features (including study design, analysis, biomarkers of exposure, and health outcome results) of important new studies are summarized in tables in the Annex for this Chapter 6 (Annex AX6).

In the main body text discussion, emphasis is placed on (1) new studies employing standardized methodological analyses for evaluating Pb effects across different study populations and providing overall effect estimates based on combined analyses of information pooled across different cohort groups; (2) meta-analyses of individual studies conducted in various study populations; (3) studies assessing Pb health effects at current relevant levels of exposure (e.g., blood Pb levels <10 µg/dL); and (4) studies conducted in the United States. Multiple cohort studies are of particular interest and value due to their evaluation of a wider range of

Pb exposures and large numbers of observations, thus generally providing more precise effect estimates than most smaller scale studies of single cohorts. Furthermore, multiple cohort studies have the potential to provide especially valuable evidence regarding relative homogeneity and/or heterogeneity of Pb health effects relationships between different study populations. Also of particular interest in recent years are those health effects observed at the lower range of Pb exposure, as typically assessed using blood Pb levels. The potential impacts of the underlying health status of populations and cultural differences in the case of intelligence testing (one of the major health outcomes in children) also need to be accounted for in the assessment; thus, U.S. studies are emphasized over non-U.S. studies. In accordance with the emphasis placed on the Pb epidemiologic studies in this chapter, Chapter 6 Annex tables are organized by emphasis on multiple cohort studies and U.S. studies.

In the ensuing sections, epidemiologic studies of the neurotoxic effects of Pb exposure in children are discussed first, in Section 6.2. The neurotoxic effects of Pb on adults are then assessed in Section 6.3. This is followed by discussion of the renal and cardiovascular effects of Pb in Sections 6.4 and 6.5. Section 6.6 next discusses reproductive and developmental effects of Pb, Section 6.7 discusses genotoxic and carcinogenic effects of Pb, and Section 6.8 discusses Pb effects on the immune system. Lead effects on other organ systems (including the hematopoietic, endocrine, hepatic, and gastrointestinal systems) are assessed in Section 6.9. The effects of Pb on bone and teeth, as well as on ocular health, are also discussed in Section 6.9. Finally, Section 6.10 discusses methodologic considerations and summarizes key epidemiologic evidence for Pb-related health effects.

6.2 NEUROTOXIC EFFECTS OF LEAD IN CHILDREN

This section assesses epidemiologic evidence for neurotoxic effects of Pb exposure in children. First presented are studies of neurocognitive effects of Pb in children, with a focus on several prospective studies examining neurocognitive ability. Other topics include measures of academic achievement, cognitive abilities, disturbances in behavior, mood, and social conduct, measures of brain anatomical development and activity, gene-environmental interaction, and reversibility of neurodevelopmental deficits. The neurotoxic effects of environmental and occupational Pb exposure of adults are then discussed in Section 6.3.

6.2.1 Summary of Key Findings on Neurotoxic Effects of Lead in Children from 1986 Lead AQCD and Addendum, and 1990 Supplement

The 1986 Lead AQCD stated that children were particularly susceptible to Pb-induced neural damage. In particular, human infants and toddlers below 3 years of age were considered to be at special risk due to possible in utero exposure, increased opportunity for exposure because of normal mouthing behavior of Pb-containing objects, and increased rates of Pb absorption due to factors such as iron and calcium deficiencies.

Effective blood Pb levels for producing encephalopathy or death in children were noted in the 1986 Lead AQCD as starting at 80 to 100 µg/dL. Various types of neural dysfunction were stated as being evident at lower blood Pb levels. Behavioral (e.g., reaction time, psychomotor performance) and electrophysiological (e.g., altered electrophysiological patterns, evoked potential measures, and peripheral nerve conduction velocities) effects were observed at blood Pb levels as low as 15 to 30 µg/dL and possibly lower. A concentration-response relationship between blood Pb levels and IQ also was observed; a 1-2 point difference in IQ was generally seen with blood Pb levels in the 15 to 30 µg/dL range. However, Schroeder and Hawk (1987) found a highly significant linear relationship between a measure of IQ and blood Pb levels over the range of 6 to 47 µg/dL among a cohort of all African-American children of low SES, suggesting that IQ effects might be detected even at blood Pb levels below 15 to 30 µg/dL.

The 1986 Addendum also discussed the newly published results of several prospective cohort studies on the developmental effects of Pb in children. These studies improved upon previous studies by utilizing longitudinal study design that followed children from the prenatal stage, larger numbers of subjects, and better analytic techniques to more accurately measure blood Pb levels. The four prospective studies (conducted in Boston, MA; Cincinnati, OH; Cleveland, OH; and Port Pirie, Australia) reported significant associations between prenatal and postnatal blood Pb levels and neurobehavioral deficits, after adjusting for various potential confounding factors, such as maternal IQ and HOME (Home Observation for Measurement of Environment) scores (Bellinger et al., 1984; Dietrich et al., 1986; Ernhart et al., 1985, 1986; McMichael et al., 1986; Vimpani et al., 1985; Wolf et al., 1985). In these studies, the observed maternal and cord blood Pb levels were fairly low, with mean levels of ~10 µg/dL. These results led to the conclusion in the 1986 Addendum that neurobehavioral deficits, including declines in Bayley Mental Development Index (MDI) scores and other assessments of neurobehavioral

function, are associated with prenatal blood Pb exposure levels on the order of 10 to 15 µg/dL and possibly even lower, as indexed by maternal or cord blood Pb concentrations.

The 1990 Supplement updated evidence from the above-mentioned longitudinal cohort studies and summarized results from other more recent prospective cohort studies conducted in Glasgow, Scotland; Kosovo, Yugoslavia; Mexico City; and Sydney, Australia. Results from several other international cross-sectional studies also were discussed. The collective evidence from the various prospective cohort and cross-sectional studies reaffirmed the conclusions from the 1986 Addendum that neurobehavioral effects were related to blood Pb levels of 10 to 15 µg/dL and possibly lower. Further analyses of the Boston data indicated that deficits in MDI could be detected in relation to cord blood Pb levels of 6 to 7 µg/dL in children within the lower strata for SES (Bellinger et al., 1988). In the Port Pirie study, the relationship between postnatal blood Pb levels and MDI at two years of age provided little evidence of a threshold effect (Wigg et al., 1988). Restricting the analysis to children with blood Pb levels below 25 µg/dL yielded an even stronger association between integrated postnatal blood Pb and McCarthy General Cognitive Index (GCI) scores in the Port Pirie study (McMichael et al., 1988).

Impaired neurobehavioral development was associated with blood Pb measures in pregnant women, umbilical cords, and infants up to at least 2 years of age; thus, no distinction could be made as to whether this level of concern applied to only fetuses or infants or preschool-age children. The issue of the persistence of the neurobehavioral effects from low-level Pb exposure also was considered. Although the Boston and Cincinnati studies provided limited evidence suggesting that the effects of prenatal Pb exposure on neurobehavioral development were not persistent, the evidence available to support this conclusion was inadequate.

6.2.2 Introduction to Neurotoxic Effects of Lead in Children

Several major developments have occurred in Pb research on child neurodevelopment following the 1986 Lead AQCD/Addendum and the 1990 Supplement. First, there has been an attempt to broaden outcome assessments beyond neurocognitive deficits. The earlier emphasis on neurocognitive measures (e.g., MDI, GCI, IQ) in previous studies is understandable from the perspectives of the strong psychometric properties of most of these rigorously standardized measures as well as the immediate pubic health concerns. Examples of other outcomes used to assess neurodevelopment include the number of errors on tests of visual-motor integration, the

time required to complete a task assessing manual dexterity, the number of errors and false alarms on a continuous performance test, and the efficiency of short term memory. Additional neurodevelopment outcomes include those which elucidate brain-behavior relationships or the potential real life consequences of early exposure to Pb, such as academic and vocational failure and maladjustment to the daily demands of living in a complex society. Thus, epidemiologic studies of Pb neurotoxicity have been expanded to adopt measures of academic achievement, specific cognitive abilities, behavior and mood, sensory acuities, neuromotor function, and direct measures of brain anatomical development and activity. Another development has been the initiation of nutritional and pharmacological intervention studies to assess the impact of treatment on reducing blood Pb levels and preventing or moderating the degree of harm to the central nervous systems of young children. Also, in addition to blood and tooth Pb, bone Pb has emerged as a reliable biomarker of Pb exposure. The technology for the assessment of Pb in cortical (tibial) and trabecular (patellar) bone using K-shell X-ray fluorescence (XRF) has advanced to the point where it could be applied as a reliable and valid index of cumulative Pb dose in neuroepidemiologic studies (Aro et al., 1994).

In recent years, more studies have investigated the impact of blood Pb levels below 10 µg/dL on the developing brain. Average blood Pb levels in U.S. children ages one to five years decreased from ~15 µg/dL to ~3 µg/dL between 1976-1980 and 1991-1994, allowing newer studies to examine the effects of low level Pb exposure on the neurodevelopment of children (Centers for Disease Control and Prevention [CDC], 2000; Pirkle et al., 1998).

At the time of the last previous criteria review, it was recognized that estimating a threshold for toxic effects of Pb on the central nervous system entailed a number of difficulties. There is the critical question of reversibility or the persistence of Pb effects identified in infants and preschoolers into school age and later. A given effect observed at younger ages may not persist due to functional compensation or a return to a normal neuromaturational trajectory (Dietrich et al., 1990). On the other hand, insults to the human brain may persist, making it difficult to determine whether any measured insult is the result of current or past exposures. An observed effect concurrent with a measured blood Pb concentration may be the result of exposure in the child's earlier life in the womb or infancy. Another problem is that it is sometimes difficult to distinguish between neurobehavioral effects due to Pb and effects owing to the many social, economic, urban-ecological, nutritional, and other medical factors that are

known to have important effects on neurobehavioral development. Equally important is the high probability that the concentration-response relationship and even the neurobehavioral lesion associated with childhood Pb exposure may vary as a function of these cofactors (Bellinger, 1995).

In the following sections, prospective cohort studies and cross-sectional studies of neurocognitive ability published since the 1990 Supplement are first discussed. Then, studies examining the effect of Pb on a variety of neurodevelopmental outcomes, including academic achievement; specific cognitive abilities; disturbances in behavior, mood, and social conduct; sensory acuities; neuromotor function; and brain anatomical development and acuity, are discussed. This is followed by discussion of several issues involved in understanding Pb neurotoxicity in children, including gene-environment interactions, reversibility of Pb effects, times of vulnerability, and potential threshold levels for effects.

6.2.3 Neurocognitive Ability

6.2.3.1 Prospective Longitudinal Cohort Studies of Neurocognitive Ability

Several prospective longitudinal cohort studies were initiated in the 1980s because it became widely recognized that the cross-sectional study design was inadequate to address a number of research issues (U.S. Environmental Protection Agency, 1986a; World Health Organization [WHO], 1977). These longitudinal studies were characterized by serial measures of dose (blood Pb levels) spanning (in most cases) the prenatal and postnatal periods of central nervous system development, thus helping to clarify the temporal association between exposure and insult. Also, developmental assessments that extended into the school-age period were planned to determine if early Pb-associated neurobehavioral impairments were persistent. It was also determined that assessment of potential confounding factors should be comprehensive and include measures of perinatal health, nutrition, maternal consumption of other neurotoxicants during pregnancy, parental intelligence, and direct observations of parenting behavior. These studies were also characterized by very careful attention to biostatistical issues and strategies (Bellinger, 1995; Ernhart, 1995).

At the time of the 1990 Supplement, studies were underway or planned in the United States, Australia, Scotland, the former Yugoslavia, and Mexico. These cohorts differed in the source and degree of Pb exposure and in other important aspects, notably ethnicity and SES.

Nevertheless, the early results from several of these studies have been largely responsible for the emergence of the current perspective that blood Pb concentrations as low as 10 µg/dL, or perhaps even lower, may pose a risk for neurodevelopmental toxicity (Davis and Svendsgaard, 1987; U.S. Environmental Protection Agency, 1990). Most of the prospective studies underway in 1990 continued to follow their subjects into the later preschool and school age years with age-appropriate measures of intelligence. Continued follow-up of these cohorts was important due to the following: (1) greater reliability and precision of measurements attained with assessments of older children; (2) high predictability of adult intellectual functioning from measures of IQ in the older child; and (3) examination of potential effects of Pb on important abilities that cannot be easily tapped during infancy such as executive functions and higher order reasoning (McCall, 1979).

A unique aspect of this research was that most investigators agreed during the formative stages of their projects to develop somewhat similar assessment protocols (Bornschein and Rabinowitz, 1985). This has facilitated comparison of results across studies and allowed for sophisticated meta- and pooled-analyses of these data (e.g., Pocock et al., 1994; Schwartz, 1994; WHO, 1995; Lanphear et al., 2005; Rothenberg and Rothenberg, 2005).

In the following sections, further updates on the individual prospective cohort studies are presented in chronological order of study initiation. The prospective cohort studies reviewed are summarized in Annex Table AX6-2.1. Results of the meta- and pooled-analyses are presented later in this section.

6.2.3.1.1 Boston Study

In the 1986 Addendum, the most advanced investigation at that time was the Boston Prospective Study (Bellinger et al., 1984). The subjects were 216 middle-to upper-middle-class Boston children, 90% of whom had cord blood Pb levels below 16 µg/dL (maximum 25 µg/dL). The children in this cohort were generally of high SES standing. While this might limit generalization of results to a wide population, this study enhanced the ability to isolate the effect of low level Pb exposure on cognitive function, as there were no associations between cord blood Pb level and several indicators of social disadvantage (e.g., receipt of public assistance, lower educational achievement, unmarried) in this highly selected subsample. Cord-blood Pb levels in the “high” group (mean 14.6 µg/dL) were associated with lower covariate-adjusted scores on the

Mental Development Index (MDI) of the Bayley Scales of Infant Development (BSID) at 6 months of age. It was concluded that although lower level Pb exposure in utero may result in delays in early sensorimotor development, the Boston results did not allow estimation of the persistence of these effects nor the public health significance of the findings.

In the 1990 Supplement, particular attention was focused on the Boston study, which was among the more mature in terms of follow-up (Bellinger et al., 1987, 1991). With respect to the effects of cord blood Pb concentrations on MDI assessed longitudinally from 6 to 24 months, the Pb-associated deficits were evident across the entire range of blood Pb levels starting at 10 µg/dL, which reinforced the previous designation of 10 to 15 µg/dL as a blood Pb of concern for early neurodevelopmental deficits. At ~5 years of age, significant associations of McCarthy GCI with the cord blood Pb level (effect estimates not provided) and concurrent blood Pb level (−2.26 points [95% CI: −6.0, 1.4] per one unit increase in ln blood Pb) were not observed, but the blood Pb level at 2 years of age (mean 6.8 µg/dL [SD 6.3]) was significantly associated with lower scores (−2.95 points [95% CI: −5.7, −0.2]). Boston investigators also examined the relationship between Pb measured in shed deciduous teeth obtained from 102 children in their cohort (mean 2.8 ppm [SD 1.7]) and GCI at 5 years of age. Prior to covariate-adjustment, there was a very strong and significant relationship amounting to a decrement of 10.04 points (95% CI: 2.6, 17.4) in GCI for each unit increase in ln dentine Pb. However, in the multivariable analysis, the effect estimate for the tooth Pb coefficient decreased to −2.51 points (95% CI: −10.2, 5.2) per unit increase in ln dentine Pb.

Since the 1990 Supplement, the Boston investigators reexamined 148 of their subjects at 10 years of age with the Wechsler Intelligence Scale for Children-Revised (WISC-R) and other neurobehavioral assessments (Bellinger et al., 1992). They examined the association of WISC-R scores at 10 years of age with blood Pb concentrations in the cord blood and at 6 months, 12 months, 18 months, 24 months, 57 months, and 10 years. Only blood Pb levels at 24 months were significantly associated with full scale and verbal IQ and marginally associated with performance IQ, after adjusting for HOME score, maternal age, birth weight, and maternal IQ. The integrated average blood Pb level in this cohort over the first 2 years was 7.0 µg/dL (range 4-14 µg/dL). An increase of 10 µg/dL in blood Pb level at age 2 was associated with a decrement of 5.8 points (95% CI: 1.8, 9.9) in full scale IQ. These findings indicated that children's performance was much more strongly associated with blood Pb levels at age 2 than

with blood Pb levels at other ages. It is unclear whether this reflects (1) a special vulnerability of the nervous system during this period; (2) the typical peaking of blood Pb levels in the second year; or (3) random chance.

A reanalysis involving the total Boston cohort that employed nonparametric smoothing revealed that the inverse association persisted at blood Pb levels below 5 µg/dL (Schwartz, 1994). Bellinger and Needleman (2003) reanalyzed data on 48 children whose measured blood Pb concentrations never exceeded 10 µg/dL. Reduction in full scale IQ at 10 years was significantly associated with blood Pb levels at 2 years of age following covariate adjustment. A larger deficit of 15.6 points (95% CI not presented) per 10 µg/dL increase in blood Pb levels was observed in this cohort, compared to the 5.8 point deficit observed in the entire cohort. These findings indicated that the inverse slope might be steeper at blood Pb levels below 10 µg/dL.

6.2.3.1.2 Cincinnati Study

Interim results on a partial sample of 185 subjects from a cohort of 305 were available from the Cincinnati prospective study in the 1986 Addendum and the 1990 Supplement (Dietrich et al., 1986, 1987a). The Cincinnati study investigators reported an inverse relationship between prenatal maternal blood Pb levels (mean 8.3 µg/dL) and 6 month Bayley MDI. This effect was mediated, in part, through Pb-associated reductions in birth weight and gestational maturity. A more complete analysis of the full Cincinnati cohort confirmed these interim findings (Dietrich et al., 1987b).

Further updates of the Cincinnati study appeared after the 1990 Supplement. For one, the Kaufman Assessment Battery for Children (KABC) was administered to ~260 children at 4 and 5 years of age (Dietrich et al., 1991; 1992). The principal findings at 4 years were that higher neonatal blood Pb concentrations were associated with poorer performance on all KABC subscales. However, this relationship was confined to children from the poorer families. After full covariate adjustment, few statistically significant relationships remained. At 5 years of age, postnatal blood Pb levels were associated with performance on all subscales of the KABC; however, few statistically significant relationships remained after adjustment for covariates. Nevertheless, it is of interest that, at both 4 and 5 years, the KABC subscale that assessed

visual-spatial skills was among those that remained the most highly associated with various indices of postnatal exposure following covariate adjustment.

At ~7 years, 253 children in the Cincinnati cohort were administered the WISC-R (Dietrich et al., 1993a). In this cohort, ~35% had at least one blood Pb concentration ≥25 µg/dL, whereas 95% exceeded 10 µg/dL sometime during the first 5 years of life. Postnatal blood Pb concentrations were inversely associated with full scale and performance IQ, after adjusting for HOME score, maternal IQ, birth weight, birth length, child gender, and cigarette consumption during pregnancy. Figure 6-1 presents the unadjusted and adjusted concentration-response relationship between lifetime average blood Pb concentrations and performance IQ. After covariate adjustment, a statistically significant relationship was observed between postnatal blood Pb levels at 5 and 6 years of age and full scale IQ. Postnatal blood Pb levels at nearly all ages (including the integrated average blood Pb level) were inversely associated with performance IQ. Due to the high intercorrelation among blood Pb measures taken at different time points, it was not practical to examine exposures during any given year for evidence of a sensitive neurodevelopmental period. Concurrent blood Pb levels were strongly associated with full scale IQ (−3.3 points [95% CI: −6.0, −0.6] for each 10 µg/dL increase in blood Pb level) and performance IQ (−5.2 points [95% CI: −8.1, −2.3]). A 10 µg/dL increase in lifetime average blood Pb concentration was associated with a 2.6 point (95% CI: 0.2, 5.0) decline in performance IQ.

At 15 to 17 years of age, the Cincinnati subjects were administered a comprehensive neuropsychological battery (Ris et al., 2004). Variables derived from the Cincinnati neuropsychological battery were subjected to a principal components factor analysis that yielded five factors, including a learning/IQ factor that had high loadings for the Vocabulary and Block Design subtests from the WISC-III as well as the Reading, Spelling, and Arithmetic subscales of the Wide Range Achievement Test-Revised (WRAT-R). Prenatal, Average Childhood, and 78 month blood Pb levels were used in a series of multiple regression analyses. After covariate-adjustment, there was a trend towards significance for higher blood Pb concentrations in later childhood (e.g., 78 months) to be associated with lower learning/IQ factor scores, but this was largely observed in subjects from the lower end of the SES scale in the sample. This finding is consistent with previous reports that children in the lower social strata may be more vulnerable

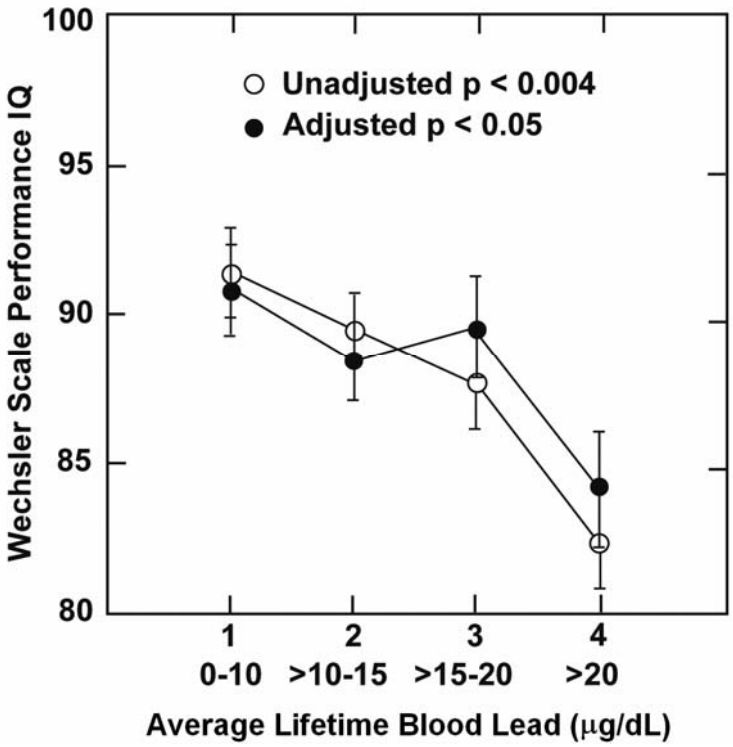

Figure 6-1. Unadjusted and adjusted relationships between average lifetime blood lead concentrations and Wechsler Scale performance IQ. Mean ± SD lifetime average blood lead concentrations within each category were as follows: 0-10 µg/dL, 7.7 ± 1.4 µg/dL (n = 68); >10-15 µg/dL, 12.3 ± 1.4 µg/dL (n = 89); >15-20 µg/dL, 17.1 ± 1.2 µg/dL (n = 53); and >20 µg/dL, 26.3 ± 5.0 µg/dL (n = 41).

Source: Dietrich et al. (1993a).

to general effects on cognitive development and learning (Bellinger, 2000; Winneke and Kraemer, 1984).

6.2.3.1.3 Cleveland Study

Early results of the Cleveland prospective study also were reviewed in the 1986 Addendum and 1990 Supplement. By selection, about half of the mothers had histories of alcohol abuse as measured by the Michigan Alcoholism Screening Test. The other women were matched controls. Through repeated interviews during pregnancy, detailed information

regarding maternal alcohol use, smoking, and the use of marijuana and other illicit drugs was obtained. While alcohol use was correlated with maternal blood Pb levels and smoking was correlated with both cord and maternal blood Pb levels, no correlations were observed between use of marijuana or other illicit drugs and any blood Pb marker. The initial cohort included 389 infants with a mean cord blood Pb level of 5.8 µg/dL (maximum 14.7). In addition to size, minor morphological anomalies, and 1- and 5-minute Apgar performances, infants were evaluated on the Brazelton Neonatal Behavioral Assessment Scale (NBAS) and part of the Graham-Rosenblith Behavioral Examination for Newborns (G-R). Of the 17 neonatal outcomes examined, the neurological soft signs assessed by G-R were associated with cord blood Pb levels ranging from 3 to 15 µg/dL following covariate adjustment (Ernhart et al., 1986). A follow-up study observed a significant effect for the neurological soft signs measure on Bayley MDI scores at 12 months (Wolf et al., 1985).

In 285 children from the original cohort, maternal and cord blood Pb levels, as well as postnatal blood Pb levels at 6 months, 2 years, and 3 years were examined in relation to Bayley MDI, Psychomotor Index (PDI), and Kent Infant Development Scale (KID) at 6 months, MDI at 1 year and 2 years, and Standford-Binet IQ (S-B IQ) at 3 years of age (Ernhart et al., 1987, 1988). The increment in variance of the IQ that can be attributed to the blood Pb level was presented as the effect estimate. Most blood Pb indices (maternal, cord, and postnatal up to 3 years) were negatively correlated with the various neurodevelopmental outcomes. However, only maternal blood Pb level at delivery (mean 6.5 µg/dL [maximum 11.8]) was found to contribute to the variance of MDI, PDI, and KID scores at 6 months after adjustment for various covariates, including HOME score, maternal IQ, parent education, race, medical problems, maternal alcohol use in pregnancy, Michigan Alcoholism Screening Test score, maternal use of marijuana, and several categories of psychosocial trauma scale.

Language development also was assessed in the Cleveland cohort at 1, 2, and 3 years of age. Once again, correlations between blood Pb measures and speech/language outcomes were generally negative, but none of the relationships remained significant after covariate adjustment (Ernhart and Greene, 1990).

The relationship between blood Pb levels at 2 years of age with the MDI at 2 years, S-B IQ at 3 years, and Wechsler Preschool and Primary Scale of Intelligence (WPPSI) test at 4 years and 10 months of age was further examined (Greene et al., 1992). The effect estimates ranged

from −11.2 to −14.6 point declines (95% CI not provided) in IQ scores for the three tests with an increase in 2-year blood Pb from 10 to 25 µg/dL. After adjusting for the various covariates, effect estimates decreased to −0.36 to −1.79 point declines for the same incremental change in blood Pb levels.

The associations between dentine Pb and IQ scores were also examined in this cohort (Greene and Ernhart, 1993). In 164 children, shed deciduous incisors were collected between ages 5 and 7 years. Circumpulpal dentine Pb levels were found to be significantly associated with full scale, verbal and performance IQ, assessed using the WPPSI test, at 4 years and 10 months, after adjustment for various covariates except for HOME score. After additional adjusting for HOME score, the effect estimates for all three IQ measures diminished, but remained statistically significant for verbal IQ ($p = 0.01$) and marginally significant for full scale IQ ($p = 0.06$). An increase in dentine Pb from the 10th percentile to the 90th percentile level (13.5 µg/g to 129.4 µg/g) was associated with a 6.0 point (95% CI: 1.4, 10.6) decrease in verbal IQ and a 4.5 point (95% CI: −0.2, 9.2) decrease in full scale IQ. Sensitivity analyses indicated that the estimated Pb effect was smaller in magnitude when measurement error was ignored. These findings using dentine Pb provide stronger evidence of inverse associations between Pb exposure and IQ scores compared to the previous analyses of this cohort, which indicated that blood Pb levels were generally not associated with cognitive outcomes after covariate adjustment.

6.2.3.1.4 Port Pirie, Australia Study

Preliminary results from the Port Pirie, Australia study also were described in the 1986 Addendum (Vimpani et al., 1985). Lower Bayley MDI scores at 2 years from 592 children were significantly associated with higher integrated postnatal blood Pb levels (~20% of the sample had blood Pb levels >30 µg/dL at the time of assessment), but not with maternal prenatal, delivery, or cord blood Pb levels. Results of this interim analysis were interpreted with caution, since important covariates such as maternal IQ and HOME scores were not available for the entire cohort at the time of the analyses.

The Port Pirie cohort study had reported results out to 4 years when the 1990 Supplement was released (McMichael et al., 1988). Following adjustment for covariates, Pb concentrations at most postnatal sampling points as well as an integrated average for the 4-year postnatal period

were significantly and inversely associated with scores on the McCarthy Scales of Children's Abilities. The GCI scores declined by ~4.5 points (95% CI: 0.2, 8.8) for a doubling in blood Pb levels. Similar deficits occurred in the perceptual-performance and memory scores. The integrated postnatal blood Pb levels among the 537 children in this cohort were among the highest of the prospective studies (geometric mean 19 µg/dL). However, further analyses indicated that the effects observed did not depend on children with the more extreme levels of exposure. The concentration-response relationship between blood Pb and GCI was stronger among children with blood Pb levels <25 µg/dL than it was overall.

Of all of the prospective studies of Pb and child development, the Port Pirie cohort study was probably among the best positioned to reliably detect effects of low level Pb exposure into later childhood owing to its wide range of exposure, large sample size, and lack of extremes in terms of sample social advantage or disadvantage. The WISC-R IQ test was administered to 494 children between 7 and 8 years of age (Baghurst et al., 1992). IQ scores were examined in relation to ln-transformed blood Pb concentration. Following adjustment for covariates, there was little association with pre- and perinatal Pb exposure assessments. However, significant decrements in full scale and verbal IQ were found to be associated with postnatal blood Pb levels. The estimated effect size was a loss of 3.3 points (95% CI: 0.2, 6.5) in full scale IQ and 4.0 points (95% CI: 0.7, 7.2) in verbal IQ in association with a doubling of the integrated postnatal blood Pb concentration up to three years. In light of the Cincinnati findings, it is of interest that the Block Design subtest of the WISC-R (a measure of visual-spatial abilities), exhibited the strongest association with Pb exposure. Port Pirie investigators also collected deciduous central upper incisors from 262 children in their cohort (McMichael et al., 1994). After covariate adjustment, a significant inverse association was observed between tooth Pb concentration and WISC-R full scale IQ at 7 years of age. The adjusted estimated decline in full scale IQ across the tooth Pb range from 3 to 22 µg/g (range for 90% of population) was 5.1 points (90% CI: 0.2, 10.0). Once again, the Block Design subtest was among the most highly sensitive.

Port Pirie children were assessed again at 11 to 13 years of age to examine the persistence of relationships between environmental Pb exposure and impacts on intelligence (Tong et al., 1996). At that age, Port Pirie investigators were able to recall 375 children for IQ assessments. At 11 to 13 years of age, the geometric mean lifetime average blood Pb concentration was

14.1 µg/dL. WISC-R scores were significantly and inversely associated with integrated lifetime average blood Pb concentrations out to 11 to 13 years. Later blood Pb concentrations after 3 years of age were more predictive of lower IQ. Mean full scale IQ declined by 3.0 points (95% CI: 0.1, 5.9) for a doubling of lifetime average blood Pb concentrations. The authors could find no clear evidence of a threshold level in their data.

6.2.3.1.5 Sydney, Australia Study

Unlike Port Pirie, the reports on the Sydney cohort study were consistently negative with respect to the effects of Pb exposure on neurodevelopment (Cooney et al., 1989a,b; McBride et al., 1989). In the 298 mothers and infants sampled, geometric mean blood Pb levels at delivery were 9.1 µg/dL and 8.1 µg/dL, respectively, with less than 2% in excess of 15 µg/dL. Mean postnatal blood Pb levels peaked at 16.4 µg/dL when children reached 18 months and then declined to 10.1 µg/dL at 48 months. No significant, inverse relationships were reported between prenatal or postnatal blood Pb concentrations and neurodevelopmental assessments conducted from 6 months through 4 years of age. The McCarthy Scales of Children's Abilities was administered to 207 children at 4 years of age, but no associations with blood Pb levels were observed prior to or following covariate-adjustment. As in the case of the Cleveland study, the authors noted that the HOME score was a strong contributor to the neurodevelopmental assessments at all ages. As stated in the 1990 Supplement, this raises the questions of whether Pb exposure might have covaried with HOME scores. If so, adjusting for HOME scores would reduce the statistical power by which to detect postnatal blood Pb effects on the neurocognitive measures. It is also noteworthy that the interpretation of the Sydney findings has been complicated by concerns about possible contamination of capillary blood Pb samples collected during the early phases of the investigation (Cooney et al., 1989b).

The Sydney prospective study further assessed 175 subjects that remained in the study at 7 years of age (Cooney et al., 1991). Geometric mean blood Pb concentrations peaked at 2 years of age (15.2 µg/dL). The geometric mean blood Pb level at 7 years of age was 7.7 µg/dL. The WISC-R and other neurobehavioral assessments were administered. The adjusted correlations between postnatal blood Pb levels and WISC-R scores were consistently negative but nonsignificant at the $p \leq 0.05$ level. The r value (units = SD of IQ per SD of blood Pb) for the correlation between full scale IQ and concurrent blood Pb at age 7 years was −0.06 (95% CI:

−0.20, 0.09). Sufficient data are not presented in this study to convert the correlation coefficients to a slope estimate. The correlation coefficient is not significantly different from that found by Bellinger et al. (1992) for 57-month-old children (−0.07 [95% CI: −0.23, 0.08]), or by Lanphear et al. (2005) for children aged 4.8 to 10 years (−0.20 [95% CI: −0.28, −0.12]). All correlation coefficients are for full scale IQ and concurrent blood Pb levels.

Results from this follow-up study were consistent with the investigators earlier reports of no association between blood Pb levels <15 µg/dL and developmental deficits among the Sydney cohort children. However, the authors noted that their study was not designed to examine small deficits associated with blood Pb levels at this magnitude. They reported that the size of their cohort did not provide sufficient power to detect effects less than 5%. Cooney et al. concluded that results from their study indicate that if developmental deficits do occur at blood Pb levels below 25 µg/dL, the effect size is likely to be less than 5%.

6.2.3.1.6 Mexico City Study (A)

Preliminary results of the Mexico City cohort prospective study (Rothenberg et al., 1989) were presented in the 1990 Supplement. Blood Pb levels from 42 mother-infant pairs were measured at 36 weeks of pregnancy (mean 15.0 µg/dL) and delivery (mean 15.4 µg/dL), and in the cord blood (mean 13.8 µg/dL). The Brazelton NBAS was administered to infants at 48 hours, 15 days, and 30 days after birth. None of the Pb measures were associated with the NBAS outcomes; however, several differential Pb measures (i.e., maternal blood Pb at 36 weeks of pregnancy minus cord blood Pb) were found to be associated with several outcome variables. Increases in the blood Pb of the mother during the last month of pregnancy or a cord blood Pb level higher than the mother's blood Pb level were associated with adverse changes in Regulation of States, Autonomic Regulation, and Gestation Age.

Schnaas et al. (2000) further examined the effect of postnatal blood Pb level on cognitive development in 112 children with complete data from the Mexico City study. Lead was measured in blood every 6 months from 6 to 54 months. Intellectual status was assessed with the McCarthy GCI. The purpose of the study was to estimate the magnitude of the effect of postnatal blood Pb level on the GCI and to determine how the effect varies with the time between blood Pb measurements and the neurocognitive assessments. The geometric mean blood Pb level between 24 to 36 months was 9.7 µg/dL (range 3.0 to 42.7). A number of

significant interactions were observed between blood Pb levels and age of assessment. The greatest effect was found at 48 months, with a decrease of 4.0 points (95% CI not presented) in adjusted GCI score being observed for a doubling of the 24 to 36 month blood Pb level. The authors concluded that 4 to 5 years of age (when children are entering school) appears to be a critical period for the manifestation of earlier postnatal blood Pb level effects.

In another study by Schnaas et al. (2006), the effect of prenatal Pb exposure on child development was examined. Of the 321 infant comprising the original cohort, 150 with complete data were included in the analyses. They used general linear models with random intercepts and slopes to analyze the pattern of Pb effects on full scale WISC-R IQ evaluated from 6 to 10 years of age. The geometric mean blood Pb levels during pregnancy, ages 1 to 5 years, and ages 6 to 10 years were 8.0 µg/dL (range 1-33), 9.8 µg/dL (range 2.8-36.4), and 6.2 µg/dL (range 2.2-18.6), respectively. The effect of log-transformed blood Pb levels from various time points on full scale IQ was examined. Only third trimester prenatal blood Pb levels were found to be significantly associated with IQ at age 6 through 10 years, after adjusting for potential confounders. A 3.44 point (95% CI: 1.28, 5.61) deficit in full scale IQ was observed for each natural log increment in blood Pb. In their discussion, however, the authors note that given the modest sample size and relatively low power of this study, they do not claim that Pb exposure from other developmental periods has no effect on child IQ.

6.2.3.1.7 Kosovo, Yugoslavia Study

The neurodevelopment results of a large birth cohort study of 577 children in two towns in Kosovo Province, Yugoslavia, were not available at the time of the 1990 Supplement. The study took place in Titova Mitrovica, near the site of a longstanding Pb smelter, refinery, and battery plant, and in Pristina, a less exposed community 25 miles to the south. A unique characteristic of this cohort was the high prevalence of anemia secondary to iron deficiency (34% with hemoglobin concentrations <10.5 µg/dL at 2 years of age). The investigators began providing iron-fortified multivitamin supplements to the entire cohort when the children were between 18 to 38 months of age (Wasserman et al., 1994).

Like Port Pirie, this was one of the more highly exposed cohorts. Blood Pb levels were obtained during the second trimester, from the umbilical cord at delivery, and postnatally at 6-month intervals to 90 months. At birth, geometric mean cord blood Pb levels were nearly

21 μg/dL in the smelter area (Wasserman et al., 1992). At age 2 years, geometric mean blood Pb concentrations were 35.5 μg/dL and 8.4 μg/dL among infants from Titova Mitrovica and Pristina, respectively.

Neurocognitive measures of mental abilities were administered at 2, 4, 7, and 10 to 13 years of age. Relationships between these neurocognitive outcomes and log-transformed blood Pb levels were assessed. A doubling of blood Pb levels at 2 years of age was associated with a covariate-adjusted decline of 1.6 points (95% CI: 0.2, 3.0) in Bayley MDI. Statistically nonsignificant decrements in MDI were associated with blood Pb levels measured at all other time points. Iron deficiency anemia also was an independent predictor of lower MDI (Wasserman et al., 1992). When examined at 4 years of age, the geometric mean blood Pb concentration of children from the smelter area was 39.9 μg/dL, whereas the geometric mean for children in the "unexposed" area was 9.6 μg/dL (Wasserman et al., 1994). Children were administered the McCarthy Scales of Children's Abilities. Higher prenatal and cord blood Pb concentrations were associated with lower GCI scores. Following covariate-adjustment, children of mothers with prenatal blood Pb levels >20 μg/dL scored a full standard deviation below children in the lowest exposure group (<5 μg/dL prenatal blood Pb). A statistically significant association also was observed between nearly every blood Pb measurement (at 6-month intervals since birth) and GCI. At 4 years of age, a doubling of blood Pb levels was associated with a reduction of 2.8 points (95% CI: 1.4, 4.3) on the GCI. The Perceptual-Performance subscale of the McCarthy was found to be most sensitive to Pb exposure.

When 301 children were examined at 7 years of age with the WISC-III, significant associations were observed between postnatal blood Pb concentrations and IQ, with consistently stronger associations between performance IQ and later blood Pb measures (Factor-Litvak, 1999). The adjusted intellectual loss associated with a doubling in lifetime average blood Pb was 2.7 points (95% CI: 1.7, 3.7) in full scale IQ, 2.8 points (95% CI: 1.7, 4.0) in performance IQ, and 2.1 points (95% CI: 1.1, 3.2) in verbal IQ. By 7 years, measures of iron status were no longer significantly associated with IQ.

At age 10 to 12 years, 290 subjects with complete data on exposure and covariate factors were again assessed with the WISC-III (Wasserman et al., 2003). However, in addition to well-characterized exposure histories based on serial blood Pb assessments, tibial bone Pb was also measured using ^{109}Cd based K-shell XRF (Todd et al., 2001) on a representative subsample of

167 subjects from both communities. Blood Pb and bone Pb measurements were highly correlated in Titova Mitrovica, but not in Pristina. Following covariate-adjustment, average lifetime blood Pb level was significantly and negatively related to all components of WISC-III IQ. A doubling of average blood Pb concentration was associated with a decrease in full scale, performance, and verbal IQ of 1.6 points (95% CI: 0.4, 2.8), 1.5 points (95% CI: 0.3, 2.8), and 1.5 points (95% CI: 0.3, 2.6), respectively. The relationships between bone Pb and IQ scores were stronger than those for blood Pb, at least in the more highly exposed smelter community. For each doubling of tibial bone Pb concentrations, full scale, performance, and verbal IQ decreased by an estimated 5.5, 6.2, and 4.1 points, respectively. The authors also reported that significant associations between tibial Pb concentrations and IQ scores persisted despite inclusion of blood Pb into the model. The inference drawn from these findings was that associations between bone Pb and IQ outcomes may be stronger than those between blood Pb measures and IQ.

6.2.3.1.8 Shanghai, China Study

A prospective study of low-level prenatal and postnatal exposure was initiated in 1993 by Shen et al. (1998) in Shanghai, China. Pregnant women were recruited from a maternal and child health care facility in the community. Lead levels were determined on 348 cord blood samples. The geometric mean cord blood Pb level was 9.2 µg/dL (range 1.6–17.5); 40.8% of the infants had cord blood Pb levels ≥10 µg/dL. Infants were further selected for study on the basis of their cord blood Pb concentrations—the low Pb group (n = 64) had levels <30th percentile while the high Pb group (n = 69) had levels >70th percentile. Mean cord blood Pb concentrations in the high Pb group and low Pb group were 13.4 µg/dL (SD 2.0) and 5.3 µg/dL (SD 1.4), respectively. At 3, 6, and 12 months, infants were administered the Chinese version of the BSID. Capillary blood samples were collected at each visit to ascertain levels of postnatal exposure. Mean blood Pb at 1 year of age was 14.9 µg/dL (SD 8.7) in the high Pb group and 14.4 µg/dL (SD 7.7) in the low Pb group. Postnatal blood Pb levels were not significantly different in the high and low Pb groups.

At all three ages, the Bayley MDI, but not PDI, was associated with cord blood Pb groupings following adjustment for covariates, which included a wide range of perinatal, demographic, social, and environmental factors. Postnatal blood Pb concentrations were not

associated with any Bayley measures. Differences in mean MDI between cord blood Pb groups were 3.4 points at 3 months ($p = 0.02$), 6.3 points at 6 months ($p = 0.03$), and 5.2 points at 12 months ($p = 0.03$). The early results of this prospective study are generally in accord with similar investigations in Boston, Cincinnati, and Cleveland. The authors concluded that the adverse effects of prenatal Pb exposure on early neurobehavioral development are readily discernible and stable over the first year of life.

6.2.3.1.9 Rochester Study

The Rochester prospective study, initiated in 1994, examined the relationship between blood Pb levels and IQ at 3 and 5 years of age in 172, predominantly African-American, lower SES children (Canfield et al., 2003a). Participants were enrolled when children were 5 to 7 months of age in what was originally a study of Pb dust control methods (Lanphear et al., 1999). Blood Pb concentrations were assessed at 6-month intervals until 2 years and annually thereafter. No data were available on prenatal exposure. The measure of IQ was the abbreviated Stanford-Binet Intelligence Scale-4th Edition (SBIS-4). Potential confounders assessed included gender, birth weight, iron status, HOME scores, maternal IQ, SES, and tobacco use during pregnancy.

Blood Pb concentrations in the Rochester cohort were quite low for an urban population, as this study was conducted after public health measures to reduce blood Pb levels in children were already having a dramatic impact in the U.S. population. Blood Pb levels peaked at 2 years of age (mean 9.7 µg/dL). The mean lifetime average blood Pb concentration was 7.7 µg/dL at the age of 3 years and 7.4 µg/dL at the age of 5 years. At 5 years of age, 56% of the children had a peak blood Pb concentration <10 µg/dL. Following adjustment for covariates, there were significant inverse associations with full scale IQ at both 3 and 5 years of age for all blood Pb variables, including lifetime average up to age of behavioral assessment.

The effect of Pb on IQ was estimated in all children using lifetime average, peak, concurrent, and average in infancy (6-24 months) blood Pb levels. Lead effects on IQ for the subgroup of children whose peak Pb concentration never exceeded 10 µg/dL also was estimated. The effect estimates were larger in the subsample of children with peak blood Pb concentrations <10 µg/dL compared to those for all children. For example, the overall estimate including all children indicated that an increase in the lifetime average blood Pb concentration of 1 µg/dL was

associated with a decrease of 0.46 points (95% CI: 0.15, 0.76) in IQ. In comparison, a 1 μg/dL increase in lifetime average Pb concentration was associated with a decline of 1.37 points (95% CI: 0.17, 2.56) in children with peak blood Pb level <10 μg/dL. In an accompanying editorial on the Canfield et al. (2003a) study, Rogan and Ware (2003) noted that the steepness in the concentration-response relationship below 10 μg/dL might have been influenced by 10 children with blood Pb concentrations at or below 5 μg/dL and IQs above 115. However, they added that it was unlikely that the associations reported by Canfield et al. were solely due to these values. Regression diagnostics performed by Canfield et al. identified only one potential outlier (a child who had a low IQ and low Pb concentration); however, this value was retained in all analyses, as it did not pass the discordancy test.

In the Rochester study, the relationship between children's IQ score and their blood Pb level was found to be nonlinear. A semiparametric analysis indicated a decline of IQ of 7.4 points for a lifetime average blood Pb concentration of up to 10 μg/dL, whereas for levels between 10 to 30 μg/dL a more gradual decrease of ~2.5 IQ points was estimated. The authors concluded that the most important aspect of their findings was that effects associated with blood Pb levels <10 μg/dL observed in previous cross-sectional studies (e.g., Chiodo et al., 2004; Fulton et al., 1987; Lanphear et al., 2000; see Section 6.2.3.2) were confirmed by this rigorous prospective longitudinal investigation.

6.2.3.1.10 Mexico City Study (B)

In another prospective cohort study conducted in Mexico City, Gomaa et al. (2002) examined prenatal and postnatal Pb exposure effects on the neurodevelopment of 197 children aged 2 years. The study cohort was recruited from 3 maternity hospitals in Mexico City that served a low- to moderate-income population. Lead was measured in the umbilical cord and maternal venous blood samples at the time of delivery. Maternal body burden was measured by obtaining cortical (tibial) and trabecular (patellar) bone Pb measurements using K-shell XRF within 4 weeks of delivery. At 2 years of age, the Bayley MDI and PDI were administered. The major objective of this study was to compare Pb levels in umbilical cord blood and maternal bone as independent predictors of infant mental development. Mean blood Pb concentrations in the cord blood, at 12 months of age, and at 24 months at age were 6.7 μg/dL (SD 3.4), 7.2 μg/dL (SD 2.8), and 8.4 μg/dL (SD 4.6), respectively. Mean maternal patella and tibia bone Pb levels

were 17.8 μg/g (range <1-76.6) and 11.5 μg/g (range <1-85.9), respectively. After covariate adjustment, postnatal blood Pb concentrations were not significantly associated with MDI (−0.09 points per 1 μg/dL increase in 24-month blood Pb, p = 0.72); however, Pb levels in cord blood were found to be significantly associated with lower scores on the Bayley MDI (−4.48 points [95% CI: −8.48, −0.48] per 1 unit increase in ln blood Pb). Maternal trabecular bone Pb levels also predicted lower Bayley MDI scores and poorer sensorimotor functioning in children 2 years of age independent of the cord blood Pb level. The authors concluded that higher maternal trabecular bone Pb concentrations constitute an independent risk factor for impaired mental development in infants at 2 years of age and that this is likely due to the mobilization of maternal bone Pb stores over the course of gestation.

Téllez-Rojo et al. (2006) further examined the longitudinal relationship between blood Pb concentrations <10 μg/dL and neurobehavioral development at 12 and 24 months of age. In addition to the first cohort of children that was recruited at the time of delivery, an additional cohort was recruited prenatally. A total of 294 mother-infant pairs met eligibility requirements, which included healthy neonatal status and blood Pb concentrations <10 μg/dL at 12 and 24 months of age. Umbilical blood Pb levels also were assessed. The primary outcome variables were the MDI and PDI of a Spanish version of the BSID-II at 12 and 24 months of age. Mean blood Pb concentrations were <5 μg/dL at both ages. Blood Pb levels at 12 months were not associated with MDI at 12 months of age. However, blood Pb levels at 24 months were significantly associated with 24-month MDI. An increase of one logarithmic unit in 24-month blood Pb level was associated with a decrement of 4.70 points (95% CI: 2.44, 6.97) in MDI. Findings for PDI were similar. Furthermore, in comparison to a supplemental subsample of 90 subjects with blood Pb ≥10 μg/dL, the coefficients of concurrent blood Pb for both the 24-month MDI and PDI were significantly steeper for children whose blood Pb never exceeded 10 μg/dL (p = 0.01). In children with blood Pb levels <10 μg/dL, a statistically significant decrement of 1.04 points (p < 0.01) was observed per 1 μg/dL increase in 24-month blood Pb compared to a 0.07 point increase (p = 0.84) in children with blood Pb levels ≥10 μg/dL. In addition, a steeper inverse slope was observed over the blood Pb range up to 5 μg/dL (−1.71 points per 1 μg/dL increase in blood Pb, p = 0.01) compared to the range between 5 and 10 μg/dL (−0.94 points, p = 0.12); however, these slopes were not significantly different (p = 0.34). In conclusion, a major finding of this prospective study was that a significant inverse

relationship between blood Pb concentration and neurodevelopment was observed among children whose blood Pb levels did not exceed 10 µg/dL at any age.

6.2.3.1.11 Pooled-Analyses of Prospective Longitudinal Cohort Studies

Investigators have collectively analyzed the results of multiple independent studies using the methods of meta- and pooled data analyses. A powerful approach involves pooling the raw data from several high quality studies to examine concentration-response relationships in a large sample of children with diverse sociodemographic backgrounds and levels of exposure. The studies reviewed here are summarized in Annex Table AX6-2.2.

Lanphear et al. (2005) reported on a pooled analysis of seven prospective studies that were initiated prior to 1995. The analysis involved 1,333 children with complete data on confounding factors that were essential in the multivariable analyses. The participating sites included Boston, MA; Cincinnati, OH; Cleveland, OH; Rochester, NY; Mexico City; Port Pirie, Australia; and Kosovo, Yugoslavia. A prospective cohort study conducted in Sydney, Australia was not included because the authors were unable to contact the investigators (Cooney et al., 1989b, 1991). The sample size of 175 for children at age 7 years in the Sydney cohort and the wide confidence intervals of the effect estimates, as implied by the lack of significant associations, indicate that the nonavailability of this study was unlikely to have influenced the results of the pooled analysis by Lanphear et al.

The primary outcome measure was full scale IQ measured at school age (mean age at IQ testing was 6.9 years). All children were assessed with an age-appropriate version of the Wechsler scales. Four measures of Pb exposure were examined: concurrent blood Pb (blood Pb level closest in time to the IQ test), maximum blood Pb level (peak blood Pb measured at any time prior to the IQ test), average lifetime blood Pb (mean blood Pb from 6 months to the concurrent blood Pb test), and early childhood blood Pb (defined as the mean blood Pb from 6 to 24 months). A pooled analysis of the relationship between cord blood Pb levels and IQ also was conducted in the subsample for which cord blood Pb tests were available.

Multivariate regression models were developed adjusting the effect of blood Pb for site as well as assessing ten common covariates potentially acting as confounders of the relationship between Pb and cognitive development, including HOME scores, birth weight, maternal education and IQ, and prenatal substance abuse. A thorough statistical analytic strategy was

employed to determine the linearity or nonlinearity of the relationship between blood Pb levels and full-scale IQ. Regression diagnostics also were performed to ascertain whether Pb coefficients were affected by collinearity or influential observations. The fit of all four measures of postnatal blood Pb levels was compared using the magnitude of the model R^2. The blood Pb measure with the largest R^2 (adjusted for the same covariates) was nominated a priori as the preferred blood Pb index relating Pb exposure to IQ in subsequent inspections of the relationships. The primary analysis was done using a fixed-effects model, although a mixed model treating sites as random effects was also examined. The authors further investigated the impact of any one site on the overall model by estimating the blood Pb coefficient in seven identical models, each omitting data from one of the seven cohort studies. Similar models were fitted for verbal and performance IQ as well.

The median lifetime average blood Pb level was 12.4 µg/dL (5th-95th percentile 4.1-34.8) with about 18% of the children having peak blood Pb levels <10 µg/dL. The 5th to 95th percentile concurrent blood Pb levels ranged from 2.4 to 30 µg/dL. The mean IQ of all children was 93.2 (SD 19.2) but this varied greatly between studies. All four measures of postnatal Pb exposure were highly correlated. However, the concurrent blood Pb level exhibited the strongest relationship with IQ, as assessed by R^2. Nevertheless, the results of the regression analyses for all blood Pb measures were very similar. Multivariable analysis resulted in a six-term model including log of concurrent blood Pb, study site, maternal IQ, HOME Inventory, birth weight, and maternal education.

Various models, including the linear model, cubic spline function, the log-linear model, and the piece-wise linear model, were investigated in this analysis. The shape of the dose-response relationship was determined to be nonlinear; the log-linear model was found to be a better fit for the data. Using the log-linear models, the authors estimated a decrement of 1.9 points (95% CI: 1.2, 2.6) in full scale IQ for a doubling of concurrent blood Pb. However, the IQ point decrements associated with an increase in blood Pb from <1 to 10 µg/dL compared to 10 to 20 µg/dL were 6.2 points (95% CI: 3.8, 8.6) versus 1.9 points (95% CI: 1.2, 2.6).

As shown in Figure 6-2, the individual effect estimates for the seven studies used in the pooled analysis also generally indicate steeper slopes in studies with lower blood Pb levels compared to those with higher blood Pb. The issue of greater effects observed at lower blood Pb levels will be discussed in detail in Section 6.2.13.

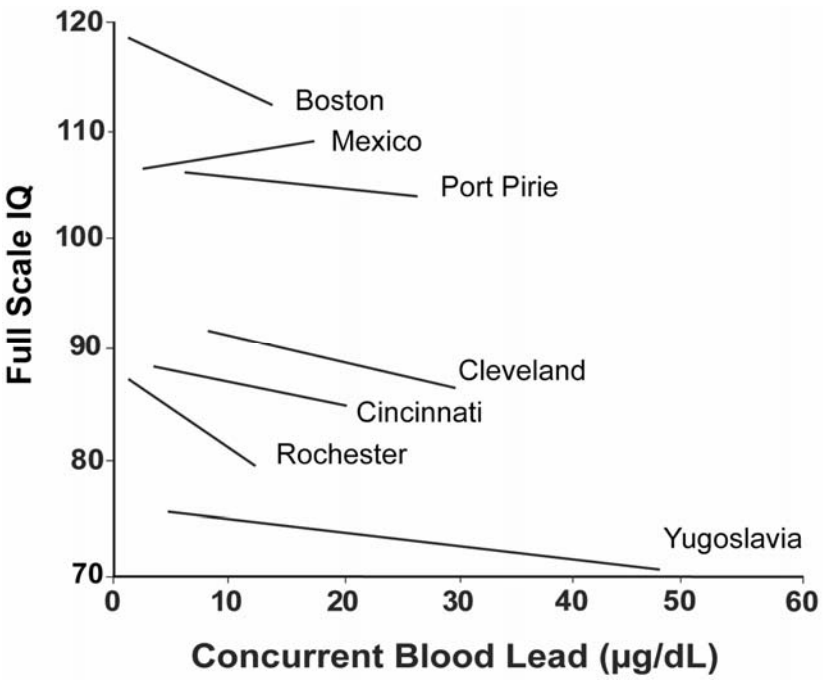

Figure 6-2. Linear models for the 7 cohort studies in the pooled analysis, adjusted for maternal IQ, HOME score, maternal education, and birth weight. The range of data shown for each study represents the 5th to 95th percentile of the concurrent blood lead level at the time of IQ testing.

Source: Lanphear et al. (2005).

Ernhart (2006) expressed the concern that one study site was driving the results and that the HOME score was not always measured with the IQ test. Other limitations were also mentioned, such as the use of capillary finger stick for the early blood Pb tests rather than venous blood Pb samples. Lanphear et al. (2006) noted that though they agree that using an early measure of the HOME inventory in the Rochester cohort was a potential limitation, excluding this cohort from the pooled analysis changed the coefficient by <3%. Sensitivity analyses reported in Lanphear et al. (2005) indicated that no single study was responsible for the estimated relationship between Pb and deficits in IQ, thus diminishing concerns about unique attributes or potential limitations for any specific sites.

In summary, the log-linear model in Lanphear et al. estimated a decline of 6.2 points in full scale IQ for an increase in concurrent blood Pb levels from <1 to 10 µg/dL. This effect estimate was comparable to the 7.4 point decrement in IQ for an increase in lifetime mean blood

Pb levels up to 10 µg/dL observed in the Rochester study (Canfield et al., 2003a), as well as other studies reviewed above.

6.2.3.2 Cross-sectional Studies of Neurocognitive Ability

Among the cross-sectional studies reviewed in the 1986 Lead AQCD and the 1990 Supplement, the most thorough and methodologically rigorous were those of Needleman et al. (1979) and Fulton et al. (1987). Needleman et al. (1979) measured Pb in the dentin of deciduous teeth in elementary school children from two Boston area communities. After statistical adjustment for a number of potential confounding factors, children in the higher tooth Pb group performed significantly less well on full scale and verbal IQ. Differences in full scale IQ between the high and low tooth Pb groups were on the order of 4.5 points.

The general population study by Fulton et al. (1987) studied 501 children aged 6 to 9 years in Edinburgh, Scotland who were at risk for Pb exposure owing to a plumbosolvent water supply and a large number of houses with Pb plumbing. Blood Pb levels averaged 11.5 µg/dL (range 3 to 34 µg/dL). Following covariate adjustment, there were statistically significant relationships between concurrent blood Pb levels and total scores on the British Ability Scale and the Quantitative and Reading subscales. Data showed a clear concentration-response relationship, with no evidence of a threshold.

More recent cross-sectional studies of neurocognitive ability are summarized in Annex Table AX6-2.3, and key studies are discussed in this section. Lanphear et al. (2000) examined the relationship between blood Pb concentrations and cognitive deficits in a nationally representative sample of 4,853 children aged 6 to 16 years children who participated in the third National Health and Nutrition Examination Survey (NHANES III). The purpose of the study was to examine the relationship between low blood Pb concentrations (especially those <10 µg/dL) and two subtests of the WISC-R, Block Design (a measure of visual-spatial skills) and Digit Span (a measure of short-term and working memory). Academic achievement tests also were administered but are discussed in a later section (see Section 6.2.4). A number of potential confounders were assessed and included in multivariable analyses, including gender, racial/ethnic background, child's serum ferritin level, serum cotinine level, region of country, marital status and education level of primary caregiver, and a poverty index ratio (the ratio of total family income, as reported by the adult informant, to the federal poverty level for the year

of the interview). Other potential confounders, such as in utero and postnatal exposure to tobacco smoke, birth weight, and admission to the neonatal intensive care unit, were only available for children between 6 and 11 years of age. Therefore, the authors conducted a secondary analysis of the data on these children to verify that inclusion of these potentially important variables did not alter the findings of the main analysis using the larger sample.

The geometric mean blood Pb level for children in the study sample was 1.9 μg/dL (SE 0.1). Only 2.1% of the NHANES III sample in this analysis had blood Pb concentrations ≥10 μg/dL. In multivariate analyses, a significant covariate-adjusted relationship was found between blood Pb level and scores on both WISC-R subtest for all children as well as among those children with blood Pb levels <10 μg/dL. Blood Pb concentration also was significantly associated with Block Design when the multivariate analysis was restricted to children with blood Pb levels <7.5 μg/dL. For a 1 μg/dL increase in blood Pb level, Block Design scores declined by 0.10 points (SE 0.04) for all children, 0.13 points (SE 0.06) for children with blood Pb levels <10 μg/dL, and 0.11 points (SE 0.06) for children with blood Pb levels <7.5 μg/dL. The authors concluded that deficits in intellectual functioning were associated with blood Pb levels <10 μg/dL; however, it is not clear whether the cognitive deficits observed were due to Pb exposure that occurred during early childhood or were a function of concurrent exposure. Nevertheless, concurrent blood Pb levels likely reflected both ongoing exposure and preexisting body burden. It should be noted that while a large number of potential confounding factors were controlled in these analyses, no data on maternal IQ or direct observations of caretaking quality in the home were available. The study did, however, control for the poverty index ratio and education level of the primary caregiver which may have served as surrogates for maternal IQ or the HOME score.

Chiodo et al. (2004) studied the relationship between blood Pb concentrations and IQ, assessed using WISC-III at 7.5 years of age in a sample of 237 African-American inner-city children from Detroit, MI. This cohort was derived from a larger study of the effects of prenatal alcohol exposure on child development. However, ~83% of children for whom blood Pb levels were obtained had either low or no gestational exposure to alcohol. Blood Pb levels were low, with a mean of 5.4 μg/dL (SD 3.3, range 1-25). Following adjustment for a wide range of covariates (including drug and alcohol exposure, HOME scores, SES status, and perinatal health among others), there was a statistically significant association between blood Pb concentrations

and full scale, verbal and performance IQ, with the strongest relationship observed for performance IQ. Significant effects of Pb on full scale and performance IQ were still evident at blood Pb <7.5 µg/dL. Nonparametric smoothing analyses confirmed that these effects were linear in nature.

Kordas et al. (2004, 2006) examined the relationship between Pb exposure and various indices of psychometric intelligence in a cohort of 602 first grade children attending public schools in Torreón, a highly industrialized city in northern Mexico. The mean blood Pb concentration was 11.5 µg/dL (SD 6.1). Approximately half of the children had blood Pb levels <10 µg/dL, and only 20% of the subjects had blood Pb levels >15 µg/dL. Subjects were administered Spanish versions of the Peabody Picture Vocabulary Test-Revised (PPVT-R), the Cognitive Abilities Test, and subtests of the WISC-R (Coding, Digit Span, and Arithmetic subtests). Letter and Number Sequencing tests (adapted from the Trail Making Test, Trails A) also were administered. After adjustment for sociodemographic variables, anemia, iron status, and growth, higher blood Pb levels were significantly associated with poorer performance on the PPVT, WISC-R Coding, and Number and Letter Sequencing. Segmented linear regressions revealed steeper slopes at lower blood Pb levels. Significant Pb effects were observed only for the segments defined by a concurrent blood Pb concentration <10 to 14 µg/dL. The authors acknowledged that a major limitation of their study is the lack of earlier measures of Pb exposure and nutritional status, and information on potentially confounding variables such as parental intelligence and quality of caretaking in the home.

Walkowiak et al. (1998) conducted a cross-sectional study examining relationships between low-level Pb and mercury (Hg) exposure and various measures of neurocognitive and neuromotor functioning in 384 children aged 6 years in three German cities. Blood Pb was measured at the time of testing and Hg burden was estimated from urine samples. As their measure of IQ, two subtests of the German WISC, Vocabulary and Block Design were administered. These subtests were treated separately as well as a summed index, which served as a surrogate for full scale IQ. Blood Pb concentrations were low (geometric mean 4.3 µg/dL [95th percentile 8.9]). Following covariate-adjustment, Vocabulary and the combined index, but not Block Design, exhibited negative associations with blood Pb of statistical or borderline statistical significance; but no associations were observed for Hg. The authors concluded that these findings roughly correspond with those of other studies that find Pb effects exposure on

measures of intelligence at blood Pb levels <10 µg/dL. However, they also cautioned that some important covariates and potential confounding variables were not measured, including parental IQ and home environment (e.g., HOME score).

The relationship between blood Pb levels and intelligence also was examined in 533 Saudi Arabian school girls aged 6 to 12 years (Al-Saleh et al., 2001). The Beery-Visual-Motor Integration (Beery-VMI) test and Test of Non-Verbal Intelligence were used as measures of intelligence. The mean blood Pb level was 8.11 µg/dL (SD 3.50), with 69% having a blood Pb level <10 µg/dL. After adjusting for various factors, including family income and parental education, a significant inverse relationship was observed for blood Pb and Beery-VMI. However, unlike various other studies, most notably the seven study pooled analysis by Lanphear et al. (2005), that observed larger effects at lower blood Pb levels, a significant association between blood Pb and Beery-VMI was not observed when data were restricted to those with blood Pb levels <10 µg/dL.

The cross-sectional studies examining the effect of Pb on neurocognitive abilities varied widely in study location, population, age of testing, and outcomes measured. These studies found that blood or tooth Pb levels were significantly associated with declines in intelligence and other neurocognitive outcomes. In general, these associations were consistently observed in studies with mean blood Pb levels <10 µg/dL.

6.2.3.3 Meta-analyses of Studies of Neurocognitive Abilities

Several meta-analyses of studies investigating associations between Pb exposure and neurocognitive abilities included results from both prospective cohort studies and cross-sectional studies. The studies reviewed here are summarized in Annex Table AX6-2.2. Needleman and Gatsonis (1990) conducted a meta-analysis of 12 studies that used multiple regression techniques to assess the relationship between Pb levels in tissues (blood or teeth) while adjusting for potentially confounding variables. Studies were weighted based on sample sizes, which ranged from 75 to 724 children. The authors divided studies into two groups according to the type of tissue analyzed for Pb (blood or teeth). Joint p-values and average effect sizes as measured by partial correlation coefficients were calculated using two different methods by Fisher and by Mosteller and Bush (Rosenthal, 1984). The joint p-values for the blood Pb studies were <0.0001 for both methods, whereas joint p-values of <0.0006 and <0.004 were obtained for tooth Pb

studies. The partial correlations ranged from −0.27 to −0.0003. Sensitivity analyses revealed that no single study was responsible for the significance of the final findings. The authors concluded that the hypothesis that Pb lowers children's IQ at relatively low Pb exposure dose was strongly supported by their quantitative analysis.

Another meta-analysis conducted by Schwartz (1994) took a different approach. Only studies relating blood Pb to IQ were chosen for quantitative review, since the concentration of Pb in the bloodstream is the main index of Pb exposure typically used as the basis for public health policy. Three longitudinal and four cross-sectional studies relating blood Pb to IQ were examined. Furthermore, while the work of Needleman and Gatsonis (1990) essentially involved combining partial correlations, the measure of effect used in the Schwartz analysis was the predicted change in full scale IQ as blood Pb increased from 10 to 20 µg/dL. For the prospective longitudinal studies, blood Pb levels at 2 years of age or average blood Pb levels up to 3 years of age were used in the analysis. This approach by Schwartz may be related to the belief at the time of the analysis that blood Pb levels during the first 3 years of life were the most critical in determining the severity of neurodevelopmental toxicity. The exclusion of blood Pb levels from other time points may be at issue, given that it now appears that later blood Pb levels may be more predictive of mental deficits (Baghurst et al., 1992; Canfield et al., 2003a; Chen et al., 2005; Dietrich et al., 1993a; Factor-Litvak et al., 1993). Studies were weighted by the inverse of the variances using a random-effects modeling procedure. The estimated decrease in IQ for an increase in blood Pb from 10 to 20 µg/dL was 2.6 points (95% CI: 1.8, 3.4). Sensitivity analyses indicated that the results were not determined by any individual study. Effect estimates were similar for longitudinal and cross-sectional studies. In another analysis, studies with mean blood Pb concentrations <15 µg/dL and >15 µg/dL had estimated effect sizes of −3.23 points (95% CI: −5.70, −0.76) and −2.32 points (95% CI: −3.10, −1.54), respectively. When the study with the lowest mean blood Pb level was examined in greater detail using nonparametric smoothing, no evidence of a threshold was found down to a blood Pb of 1 µg/dL.

Pocock et al. (1994) conducted a review of the epidemiologic evidence for Pb effects on IQ that included a meta-analysis. For the meta-analysis, the fixed-effect method described by Thompson and Pocock (1992) was used. Five prospective and 14 cross-sectional studies (with both tooth and blood Pb measures) were included. For consistency, only blood Pb levels at or around 2 years of age were considered for the prospective studies. Their overall conclusion was

that a doubling of blood Pb levels from 10 to 20 µg/dL or of tooth Pb from 5 to 10 µg/g was associated with an average estimated IQ deficit of about 1 to 2 points.

Other earlier meta-analyses of Pb-IQ studies have been published but are not reviewed here, because later work greatly extended these efforts and included more studies, rendering those analyses outdated (Needleman and Bellinger, 1988; Schwartz, 1985; Thacker et al., 1992). The meta-analyses of studies investigating the effect of Pb on neurocognitive ability all consistently observed significant associations between blood or tooth Pb levels and decrements in IQ. Also, the Schwartz (1994) analysis found no evidence of a threshold at blood Pb levels below 10 µg/dL.

6.2.4 Measures of Academic Achievement

Relatively little data are available on the relationship between Pb exposure and objective measures of academic achievement. A few earlier studies reported an inverse relationship between Pb exposure and reading skills (Fergusson et al., 1988a; Fulton et al., 1987; Yule et al., 1981). Since the 1990 Supplement, more studies have focused on the practical consequences of childhood Pb exposure by including measures of academic performance in their batteries. Studies reviewed in this section are summarized in Annex Table AX6-2.4.

Using NHANES III data, Lanphear et al. (2000) examined the relationship between blood Pb levels and a standardized measure of academic achievement in 4,853 children aged 6 to 16 years. This cohort was previously described in Section 6.2.3.2. Subjects were administered the Arithmetic and Reading subtests of the Wide Range Achievement Test-Revised (WRAT-R). The WRAT-R Arithmetic subtest includes oral and written problems ranging in level from simple addition to calculus, while the Reading subtest assesses letter recognition and word reading skills. The geometric mean blood Pb concentration was 1.9 µg/dL. Only 2.1% of the subjects had blood Pb levels ≥10 µg/dL. Multiple linear regression revealed a 0.70 point (95% CI: 0.37, 1.03) decrement in arithmetic scores and a 0.99 point (95% CI: 0.62, 1.36) decrement in Reading scores for each 1 µg/dL increase in concurrent blood Pb concentration ($p < 0.001$). In the next phase of the analysis, the adjusted relationship between performance on WRAT subtests and blood Pb concentration for children with blood Pb levels <10 µg/dL, <7.5 µg/dL, <5 µg/dL, or <2.5 µg/dL were carried out. Statistically significant inverse relationships between blood Pb levels and performance for both Reading and Arithmetic subtests were found for

children with blood Pb concentrations <5 μg/dL. Secondary analysis limited to younger children with data on all covariates did not alter findings from the main analysis. The authors concluded that results of these analyses suggest that deficits in academic skills are associated with blood Pb levels <5 μg/dL. The potential limitations of this study, including the lack of information on previous blood Pb levels, maternal IQ, and caretaking quality in the home, were discussed in Section 6.2.3.2.

Needleman et al. (1990) reexamined the Chelsea and Somerville, MA cohort of first and second graders recruited in the 1970s (Needleman et al., 1979). Of the original 270 children, 132 were recalled. Relationships between concentration of Pb in shed deciduous teeth and neurobehavioral deficits had persisted into late adolescence. Subjects with dentin Pb levels >20 ppm were at higher risk of dropping out of high school (adjusted odds ratio of 7.4, [95% CI: 1.4, 40.7]) and of having a reading disability (adjusted odds ratio of 5.8 [95% CI: 1.7, 19.7]). Higher dentin Pb levels were also significantly associated with lower class standing, increased absenteeism, and lower vocabulary and grammatical reasoning scores on the Neurobehavioral Evaluation System (NES). The authors concluded that undue exposure to Pb had enduring and important effects on objective parameters of success in real life.

Bellinger et al. (1992) administered a battery of neuropsychological tests to 148 Boston Lead Study cohort children at age 10 years. The short-form of the Kaufman Test of Educational Achievement (KTEA) was administered in addition to IQ studies. The KTEA assesses reading, math, and spelling skills. The primary outcome was the Battery Composite Score. As previously indicated, exposures in this cohort were low (with a peak mean blood Pb at 18 months of only 7.8 μg/dL [SD 5.7]), and the cohort consisted of high-SES White children from intact families with college-educated parents. Average KTEA scores in this cohort were about one standard deviation above the population mean. Nevertheless, postnatal blood Pb levels measured at virtually all ages were significantly associated with lower KTEA Battery Composite Scores. However, after covariate-adjustment, including full scale IQ in the model, only blood Pb levels at 24 months of age were significantly predictive of lower academic achievement. Over the range of ~0 to 25 μg/dL, Battery Composite scores declined by ~8.9 points (95% CI: 4.2, 13.6) for each 10 μg/dL increase in 24-month blood Pb. The specific subscales of the KTEA that were most significantly associated with Pb were Spelling and Math. Within the Math subscale, Pb appeared to be more strongly associated with performance on the

advanced quantitative Concepts/Applications items than on computation. The associations between these early measures of low level Pb exposure and achievement were significant even after adjustment for IQ, suggesting that Pb-sensitive neuropsychological processing and learning factors not reflected in indices of global intelligence may contribute to reduced performance on academic tasks.

Leviton et al. (1993) evaluated the relationship between pre- and postnatal Pb exposure and academic problems in ~2,000 children born in one Boston hospital between 1979 and 1980 using the Boston Teacher Questionnaire (BTQ). A teacher provided an assessment of each child's academic functioning when the child reached the age of 8 years. Mean umbilical cord blood Pb was 6.8 µg/dL and mean tooth (dentin) Pb concentration was 2.8 µg/g. There was limited information on covariate factors. Still, after adjustment for potential confounding variables, elevated dentin Pb levels were associated with statistically significant reading and spelling difficulties as assessed by the BTQ among girls. The authors concluded that their findings supported the case for Pb-associated learning problems at levels prevalent in the general population. However, they added that the inability to assess child-rearing quality in this questionnaire study conducted by mail limits the inferences that can be drawn from the findings.

Rabinowitz et al. (1992) examined the relationship between tooth Pb concentrations and scores on BTQ clusters in 493 Taiwanese children in first through third grade. Mean Pb levels in incisors were 4.6 µg/g (SD 3.5). Factors associated with Pb and BTQ scores included 13 variables measuring perinatal, familial, and economic parameters. Prior to adjustment for covariates, girls in this sample with higher exposures to Pb showed a borderline significant trend for reading difficulties, whereas boys displayed significantly increased difficulties with respect to activity levels and task attentiveness. In multiple logistic regression models, tooth Pb terms failed to achieve statistical significance. The authors concluded that Pb levels found in the teeth of children in their Taiwanese sample were not associated with learning problems or syndromes as assessed by the BTQ.

Fergusson et al. (1993) examined the relationship between dentin Pb levels in shed deciduous teeth at 6 to 8 years and measures of academic attainment and classroom performance in a birth cohort of over 1,200 New Zealand children enrolled in the Christchurch Health and Development Study when they reached 12 to 13 years of age. This study was an extension of earlier work in these children indicating a relationship between low Pb levels and deficits in

academic skills around the age of 8 years (Fergusson et al., 1988a). Average dentine Pb levels in the cohort were 6.2 μg/g (SD 6.2). Measures of academic performance included word recognition from the Burt Reading Test, reading comprehension from the Progressive Achievement Test, a general measure of scholastic skills based on children's scores on the Test of Scholastic Abilities, and teacher ratings of classroom performance in reading, written expression, and mathematics. Following adjustment for a wide range of covariates (including residence in potentially Pb-hazardous housing), dentin Pb levels were significantly associated with virtually every formal index of academic skills and teacher ratings of classroom performance. Statistical evaluations included a multivariate analysis of all 12 regression equations simultaneously using LISREL modeling methods. This conservative analysis clearly showed that the probability of observing these results under the null hypotheses that Pb was unrelated to all covariate-adjusted test outcomes was extremely small. In an adjunct analysis, Fergusson and Horwood (1993) examined low-level Pb exposure effects on the growth of word recognition in this cohort from 8 to 12 years of age, using growth curve modeling methods. After adjustment for potential confounding variables, children with dentin Pb levels ≥8 μg/g displayed significantly slower growth in word recognition abilities with no evidence of catch up. The authors concluded that these results were consistent with their earlier analyses and suggest that early exposure to very low levels of Pb result in small but detectable and enduring deficits in children's cognitive abilities.

Academic achievement in relationship to Pb was reexamined in the New Zealand cohort when subjects reached 18 years of age (Fergusson et al., 1997). The sample at 18 years consisted of 881 subjects, or ~70% of the original cohort. Measures of educational achievement included the Burt Reading Test, number of years of secondary education, mean number of School Certificate passes (based on results of national examinations), and leaving school without formal qualifications (analogous to failure to graduate from high school in the United States). As in previous analyses, a wide range of potentially confounding socioherediatry factors were measured and controlled for in multivariable analyses, which included both linear and logistic regressions. Prior to and following covariate adjustment, there were statistically significant concentration-response relationships between dentin Pb concentrations and lower reading test scores, having a reading level of less than 12 years, failing to complete 3 years of high school, leaving school without qualifications, and mean number of School Certificates subjects passed.

The authors concluded that their results are consistent with the view that there is a relationship between early low-level Pb exposure and later educational outcomes. The late results of the New Zealand studies confirm the Needleman et al. (1990) findings in a cohort with lower levels of environmental Pb exposure.

Wang et al. (2002a) examined the relationship between blood Pb levels and class ranking in 934 third graders living in an urban industrial area of Taiwan. The outcome variables were grades for Chinese (reading and writing), Mathematics, History and Society, and Natural Science. To avoid the impact of teacher's bias in grading criteria, the authors converted the children's grades into class rankings. A limited number of potentially confounding factors were measured, including maternal education and father's SES. Mean blood Pb level was 5.5 µg/dL (SD 1.89). In multiple regression analyses adjusting for gender, maternal education, and father's SES, blood Pb was significantly associated with lower class ranking in all academic subjects. The major shortcoming of this cross-sectional study is the lack of control for potentially important confounding factors such as parental intelligence. However, the strength and consistency of the reported relationships suggest that relatively low level Pb exposure may play a role in lowering academic performance.

Al-Saleh et al. (2001) studied the association between blood Pb levels and academic achievement in 533 girls aged 6 to 12 years in Riyadh, Saudi Arabia. At the time of this study leaded gasoline was still in wide use. The measure of academic achievement was based on the class ranking of each student as assessed by the teacher. A large number of confounding variables were considered, including growth parameters and various assessments of SES, health status, geographical location and family structure. The mean blood Pb in the cohort was 8.11 µg/dL (SD 3.5). Following covariate adjustment, there was a statistically significant relationship between higher blood Pb levels and lower class rank percentile subscales. When multiple regression models were fitted to a subset of students with blood Pb levels <10 µg/dL, class rank percentile continued to show a statistically significant association with blood Pb levels.

Kordas et al. (2006) examined the relationship between blood Pb levels at 7 years of age, and math achievement and vocabulary in 594 second graders living near a metal foundry in Torreón, Mexico. The mean blood Pb level was 11.4 µg/dL (SD 6.1). Following adjustment for covariates measuring other well-documented predictors of cognitive functioning as well as

concurrent arsenic exposure, blood Pb concentrations were statistically significantly related to poorer math and vocabulary scores. Furthermore, in segmented regression analyses, the slopes for the association of blood Pb with vocabulary and math scores were both significantly steeper below 10 μg/dL than above.

The results of these studies strongly suggest that Pb exposure can affect the academic performance of children. Consistent associations also were observed in cohorts of children with mean blood Pb levels below 10 μg/dL.

6.2.5 Measures of Specific Cognitive Abilities

Outcomes of specific cognitive abilities, in particular, the domains of Attention and Executive Functions, Language, Memory and Learning, and Visuospatial Processing have been examined in some detail in recent studies. These studies are summarized in Annex Table AX6-2.5.

In the aggregate, studies suggest that Pb exposure impairs a child's ability to regulate attention and to engage several related higher-order cognitive processes that have come to be termed "executive functions." Executive functions refer to strategic planning, control of impulses, organized search, flexibility of thought and action, and self-monitoring of one's own behavior—activities that help the subject maintain an appropriate mental set in order to achieve an immediate or future goal (Spreen et al., 1995). In some earlier studies, increased Pb exposure was found to be associated with a higher frequency of negative ratings by teachers and/or parents on behaviors such as inattentiveness, impulsivity, distractibility, and less persistence in assigned tasks, as well as slow psychomotor responses and more errors on simple, serial, and choice reaction time tasks (e.g., Hatzakis et al., 1989; Hunter et al., 1985; Needleman et al., 1979; Raab et al., 1990; Winneke et al., 1990). The concept that Pb may impact executive functions in particular is biologically plausible. The prefrontal cortex is highly innervated by projections of neurons from the midbrain and has the highest concentration of dopamine of all cortical areas. Dopamine plays a key role in cognitive abilities mediated by the prefrontal cortex. It has been known for some time that the dopamine system is particularly sensitive to Pb, based upon studies of rodents and nonhuman primates (Cory-Slechta, 1995).

Bellinger et al. (1994a) examined a portion of the original Chelsea and Somerville cohorts at 19 to 20 years of age. The main neurobehavioral outcomes used were scores on a battery of

attentional measures assembled by Mirsky (1987). Higher tooth Pb concentrations were significantly associated with poorer scores on the Focus-Execute and Shift factors of the battery, leading the authors to conclude that early Pb exposure may be associated with poorer performance on executive/regulatory functions thought to depend on frontal or prefrontal brain regions.

Stiles and Bellinger (1993) administered a neuropsychological battery of tests to 10-year-old children in the Boston Lead Study cohort. A large number of assessments were made and, as the authors acknowledge, the number of significant associations was about equal to those that would be expected by chance. However, as in previous studies, tasks that assess attentional behaviors and executive functions tended to be among those for which Pb was a significant predictor of performance. For example, higher blood Pb concentrations at 2 years were significantly associated with (a) lower scores on the Freedom from Distractibility factor of the Wechsler scales and (b) an increase in the percentage of preservative errors on the Wisconsin Card Sorting Test and the California Verbal Learning Test. At 2 years of age, 90% of the children had blood Pb levels <13 μg/dL.

Canfield et al. (2003b) conducted a comprehensive evaluation of effects of low-level Pb exposure on executive functioning and learning in children from the Rochester Lead Study cohort at 48 and 54 months of age. The mean blood Pb level at 48 months was 6.49 μg/dL (range 1.7-20.8), with 80% of the children having a blood Pb <10 μg/dL. The authors used the Shape School Task (Espy, 1997), which requires only knowing simple shape and primary color names. However, embedded in the tasks are protocols requiring inhibition, attention switching, and a combination of inhibition and switching mental sets. Following covariate-adjustment, blood Pb level at 48 months was negatively associated with children's focused attention while performing the tasks, efficiency at naming colors, and inhibition of automatic responding. Children with higher blood Pb levels also completed fewer phases of the task and knew fewer color and shape names.

Canfield et al. (2004) also administered portions of the Cambridge Neuropsychological Testing Automated Battery (CANTAB) to 174 Rochester cohort children at ~66 months of age. Children were tested with the Working Memory and Planning CANTAB assessment protocols to assess mnemonic and executive functions. Blood Pb levels ranged from 0 to 20 μg/dL in this cohort. Following covariate adjustment, children with higher blood Pb levels showed impaired

performance on tests of spatial working memory, spatial memory span, cognitive and cognitive flexibility, and planning as indexed by tests of intradimensional and extradimensional shifts and an analog of the Tower of London task.

Ris et al. (2004) administered an extensive neuropsychological battery to 15-17 year old subjects from the Cincinnati Lead Study cohort. Besides executive functions assessed by the Wisconsin Card Sorting Test and the Rey-Osterrieth Complex Figure, other domains examined included attention, memory, achievement, verbal abilities, visuoconstructional skills, and fine-motor coordination. About 30% of the subjects had blood Pb levels ≥25 µg/dL during the first 5 years of life; and 80% had at least one blood Pb ≥15 µg/dL. A factor analysis of scores selected a priori revealed five factors that included Attention. A strong "executive functions" factor did not emerge. After covariate-adjustment, the strongest associations between Pb exposure and performance were found for factor scores derived from the Attention component, which included high loadings on variables from the Conners Continuous Performance Test. However, this relationship was restricted to males as indicated by a strong Pb-by-gender interaction. This observed gender interaction suggests that neuromechanisms sub-serving attention were affected by Pb in this cohort for boys but not for girls. This is not surprising given the heightened vulnerability of males for a wide range of developmental perturbations. For example, a substantial gender difference in the incidence of Attention Deficit/Hyperactivity Disorder (ADHD) is well established, and one could speculate that early exposure to Pb exacerbates a latent potential for such problems.

Visual-spatial skills have also been also been explored in some depth by a few studies. When investigations of Pb-exposed children have used global IQ measures and conducted subscale analyses, it has been observed that Performance IQ or subtests contributing to the performance IQ (i.e., Block Design) are frequently among the most strongly associated with biological indices of Pb exposure (Baghurst et al., 1992; Chiodo et al., 2004; Dietrich et al., 1993a; McMichael et al., 1988; Wasserman et al., 1994). Dietrich et al. (1991, 1992) have also observed that integrated measures of Pb exposure over a child's lifetime are most consistently associated with simultaneous processing abilities, cognitive functions closely associated with visual-spatial integration skills and right cerebral functioning (Kaufman and Kaufman, 1983). In addition, studies employing specific measures of visual-motor integration skills, such as the Developmental Test of Visual Motor Integration (VMI), the Bender Visual-Motor Gestalt Test,

and others, have found them to be among the most consistently associated with early Pb exposure (Al-Saleh et al., 2001; Baghurst et al., 1995; Dietrich et al., 1993b; Wasserman et al., 2000a; Winneke et al., 1990). In a follow-up of Cincinnati Lead Study cohort subjects at age 16 years, Ris et al. (2004) observed a significant association between prenatal maternal blood Pb levels and deficits in visual-spatial and constructional skills as indexed by Visual-Constructional factor scores. Variables with high loadings on this factor included scores on the WISC-III Block Design subtests and selected variables from the Rey Osterrieth Complex Figure.

Kordas et al. (2006) administered an extensive battery of tests assessing specific abilities to 594 first graders (mean blood Pb of 11.4 µg/dL) in Torreón, Mexico, the site of a metal foundry. The battery included well validated assessments of mental distractibility, sequencing skills, memory, visual spatial skills and stimulus discrimination. Following adjustment for covariates in linear regression analyses, blood Pb remained significantly associated with performance on the Sternberg Memory test. For the various tests, steeper slopes were generally observed for blood Pb levels below 10 µg/dL than above.

It is still unclear whether the domains of attention/executive functions or visual-motor integration per se are specifically sensitive to Pb. This is because there is rarely a one-to-one correspondence between performance on a focused neuropsychological test and an underlying neuropsychological process. Thus, for example, a low score on the Berry VMI may reflect singular or multiple neurobehavioral deficits, including difficulties with graphomotor control, visual perception, behavioral monitoring (impulsivity), or planning (executive functions).

6.2.6 Disturbances in Behavior, Mood, and Social Conduct

Lead effects on behavior and mood of children has been an area of recent research. Studies conducted prior to 1990 clearly pointed to behavioral problems as potential sequelae of lower level Pb toxicity in children. Several early case control studies have linked Pb to hyperactivity (David et al., 1972, 1976, 1979). Low levels of Pb in blood and/or teeth have been associated with teacher ratings of hyperactive behavior, aggression, and attention problems (e.g., Fergusson et al., 1988b; Hatzakis et al., 1985; Silva et al., 1988; Thomson et al., 1989; Yule et al., 1984). In the seminal study by Needleman et al. (1979), children with higher Pb concentrations in dentin were more likely to be rated unfavorably by teachers on the dimensions

of hyperactivity, impulsivity, and frustration tolerance. New studies reviewed in this section are summarized in Annex Table AX6-2.6.

While there is no compelling evidence that Pb exposure is directly related to ADHD, elevated blood or tooth Pb levels have been linked to behavioral features of ADHD, including distractibility, poor organization, lacking persistence in completing tasks, and daydreaming (Bellinger and Rappaport, 2002). Bellinger et al. (1994b) studied the relationship between early Pb exposure and problem behaviors in the classroom in a cohort of 1,782 children born at one hospital in Boston. Umbilical cord blood Pb levels were low (mean 6.8 µg/dL [SD 3.1]) as were tooth Pb levels (mean 3.4 µg/g [SD 2.4]). Teachers filled out the Achenbach Child Behavior Profile (ACBP), which yields both broad and narrow band scales indexing externalizing and internalizing problems. Cord blood Pb levels were not associated with the prevalence or nature of behavioral problems reported by teachers. However, tooth Pb level was significantly associated with ACBP Total Problem Behavior Scores (TPBS). TPBS scores increased by ~2 points for each log unit increase in tooth Pb. Statistically significant tooth Pb-associated increases in both externalizing and internalizing scores were also noted. Each log unit increase in tooth Pb was associated with a 1.5 point increase in scores for these broadband scales assessing under- and overcontrol of behavior. Only weak associations were seen between tooth Pb concentrations and the tendency to score in the clinically significant range on these scales. As the authors noted, it was somewhat surprising that Pb exposure was not more strongly related to externalizing behavior problems than with internalizing behavior problems. This contradicted several earlier investigations, including one by Sciarillo et al. (1992) (see Annex Table AX6-2.6). It may be that more attention has been accorded undercontrolled behaviors, because they are more readily visible and disruptive in settings such as the classroom. Therefore, internalizing problems may be part of the full spectrum of behaviors in which the developmental neurotoxicity of Pb is expressed in children. The authors also cautioned that residual confounding could not be ruled out, because of the lack of covariate information on parental psychopathology or direct observations of the family environment—a problem not unique to this particular study. Nevertheless, these findings are in accord with other studies which suggest that social and emotional dysfunction may be another expression of increased Pb exposure during the early postnatal period.

Fergusson et al. (1993) studied relationships between tooth Pb levels and inattention/ restlessness in the large national New Zealand study of over 1,000 children at 12 and 13 years of age. Mothers and teachers were asked to respond to a series of items derived from the Rutter and Conners parental and teacher questionnaires. The selected items related to the degree to which the child was restless, inattentive, easily distracted, and lacking in concentration. At each age, an index of the subject's propensity to inattentive and restless behavior was obtained by summing the total reports of attention deficit behaviors made by both teacher and parent respondents. Following adjustment for a wide range of sociodemographic and other covariate factors, a statistically significant, concentration-response relationship was observed between tooth Pb concentrations (range 1 to 12+ µg/g) and the inattention/restlessness variable. The authors concluded that their results were consistent with the view that early mildly elevated Pb levels were associated with small but long-term deficits in attentional behaviors.

As part of the 11-year follow-up of the Dunedin Multidisciplinary Health and Development Study, a longitudinal study of a birth cohort of children born in Dunedin's only obstetric hospital, blood Pb levels were measured in 579 children at age 11 years old (Silva et al., 1988). The study sample was over-representative of higher SES. The mean blood Pb level was 11.1 µg/dL (SD 4.91), with a range from 4 to 50 µg/dL (only two children had blood Pb levels >30 µg/dL). The correlations between blood Pb levels and WISC IQ variables were negative but not statistically significant. However, blood Pb levels were found to be significantly associated with increased behavioral problems as assessed by both parents and teachers, even after controlling for various factors including SES, other disadvantageous factors, maternal cognitive ability, and IQ.

Two prospective studies have also examined early Pb exposure relationships to behavioral problems as assessed by the Achenbach system. Wasserman et al. (1998) studied the relationship between Pb exposure and behavior in the Yugoslavian prospective study. The study surveyed 379 children at 3 years of age with the parent report form of the Achenbach CBCL. Following covariate adjustment, concurrent blood Pb levels were significantly associated with scores on the Destructive Behaviors CBCL subscale, although the variance accounted for by Pb was small compared to sociodemographic factors. As blood Pb increased from 10 to 20 µg/dL, CBCL subscale scores increased by ~0.5 points. The authors concluded that while statistically significant, the contribution of Pb to social behavioral problems in this cohort was

small compared to the effects of correlated social factors. Burns et al. (1999) examined the relationship between Pb exposure and children's emotional and behavioral problems at ages 11 to 13 years in the Port Pirie, Australia, cohort study. After adjusting for many confounding variables, including HOME scores, maternal psychopathology and the child's IQ, regression models showed that, for an increase in average lifetime blood Pb levels from 10 to 30 µg/dL, the externalizing behavior problem score increased by 3.5 points (95% CI: 1.6, 5.4) in boys but only by 1.8 points (95% CI: –0.1, 11.1) in girls. In contrast, internalizing behavior problems were predicted to increase by 2.1 points (95% CI: 0.0, 4.2) in girls, but by only 0.8 points (95% CI: −0.9, 2.4) in boys.

Recently, the potential role of Pb in delinquent and criminal behavior has been addressed by several investigations. Previous studies linking attention deficits, aggressive and disruptive behaviors, and poor self-regulation with Pb exposure have raised the prospect that early exposure may result in an increased likelihood of engaging in antisocial behaviors in later life.

Denno (1990) surveyed 987 Philadelphia African-American youths enrolled in the Collaborative Perinatal Project. Data were available from birth through 22 years of age. The analysis initially considered over 100 predictors of violent and chronic delinquent behavior. Repeat offenders presented consistent features such as low maternal education, prolonged male-provider unemployment, frequent moves, and higher Pb intoxication (although the level of Pb intoxication was not indicated in Denno's report). In male subjects, a history of Pb poisoning was among the most significant predictors of delinquency and adult criminality.

Needleman et al. (1996) examined relationships between Pb exposure and several measures of behavioral disturbance and delinquent behavior in subjects from the Pittsburgh Youth Study. The Pittsburgh Youth Study is a prospective study of the developmental course of delinquency (Loeber et al., 1991). The population consisted of 850 boys who were prescreened with an instrument that measured serious and potentially indictable behaviors extracted from the teachers' and parents' CBCL. Subjects who scored above the 30th percentile on the risk score and an approximately equal number of subjects randomly selected from the remainder of the distribution formed the sample (n = 503). Body burden of Pb was measured in the tibia by K-shell XRF. Measures of antisocial behavior were administered at 7 and 11 years of age, and included the Self Reported Antisocial Behavior scale (SRA), the Self Report of Delinquent Behavior (SRD), and the parents' and teachers' versions of the CBCL. Outcome data were

adjusted for a number of covariates, including mother's IQ, SES, childhood medical problems, and quality of child rearing. Parents of subjects with higher Pb levels in bone reported significantly more somatic complaints, more delinquent and aggressive behavior, and higher internalizing and externalizing scores. Teachers reported significant increases in scores on somatic complaints, anxious/depressed, social problems, attention problems, delinquent behavior, aggressive behavior, and internalizing and externalizing problems in the higher Pb subjects. At 11 years, the SRD scores of subjects were also significantly related to bone Pb levels. More of the high Pb subjects had CBCL scores in the clinical range for the CBCL subscales assessing attention problems, aggression, and delinquency. Odds ratios for these outcomes ranged from 1.5 (95% CI: 0.45, 4.9) for parental reports of aggression to 19.5 (95% CI: 8.9, 41.6) for attention problems. The authors concluded that Pb exposure was associated with an increased risk for antisocial and delinquent behavior.

Dietrich et al. (2001) reported on the relationship between early Pb exposure and juvenile delinquency in 195 subjects from the Cincinnati Lead Study. As previously noted, this is an inner-city cohort of urban children exposed to relatively high levels of Pb by virtue of their residence in older, deteriorated housing units. Relationships were evaluated between prenatal (maternal) and postnatal exposure to Pb (through serial blood Pb determinations) and measures of antisocial and delinquent behaviors (self- and parental reports) examined when the subjects were 16 or 17 years old. Parents were administered a questionnaire developed specifically for the study, while the subjects were given the SRD. A wide range of candidate covariates and confounders were examined, but the only ones predicting antisocial or delinquent behavior were birth weight, HOME scores, SES, and parental IQ. In multiple linear regression analyses, prenatal exposure was significantly associated with a covariate-adjusted increase in the frequency of parent-reported delinquent and antisocial acts, whereas prenatal and postnatal Pb exposure was significantly associated with a covariate-adjusted increase in frequency of self-reported delinquent and antisocial behaviors, including marijuana use. In order to clarify concentration-response relationships, blood Pb indices were transformed to categorical variables and least-square means were calculated from an analysis of covariance procedure. Subjects in the highest prenatal blood Pb category (>10 μg/dL) engaged in 2.3 more delinquent acts over the preceding 12 months than subjects in the lowest category (≤5 μg/dL). Using average childhood blood Pb levels, subjects in the medium (16-20 μg/dL) and highest (>20 μg/dL) category

engaged in ~1.5 more delinquent acts compared to the lowest category (≤10 µg/dL). Subjects in the highest 78-month blood Pb category (>15 µg/dL) engaged in 4.5 more delinquent acts than subjects in the lowest category (≤5 µg/dL). The authors concluded that Pb might play a measurable role in the epigenesis of behavioral problems in inner-city children independent of other social and biomedical cofactors assessed in the study.

Needleman et al. (2002) conducted a case-control study that examined Pb levels in bone of 194 adjudicated delinquents and 146 non-delinquent community control subjects recruited from high schools in the city of Pittsburgh and environs of Allegheny County, PA. Since many delinquents are not arrested or adjudicated, care was taken to ensure that unidentified delinquents did not populate the control group. Potential control subjects were excluded from the analyses if found to have a Juvenile Court record or an SRD score above the 90th percentile. Tibial bone Pb was measured by K-shell XRF. Covariates included race, parental education and occupation, presence of two parental figures in the home, number of children in the home, and neighborhood crime rate. Logistic regression analyses were used to model the association between bone Pb concentration and delinquent status. Cases had significantly higher average tibia Pb levels than controls (11.0 µg/g [SD 32.7] versus 1.5 µg/g [SD 32.1]). Stratified analyses showed this for both White and African-American subjects. Following adjustment for covariates, adjudicated delinquents were four times more likely to have bone Pb concentration >25 µg/g than controls (odds ratio of 4.0 [95% CI: 1.4, 11.1]). The effect of Pb on delinquency was found to be substantial in this study. Bone Pb level was the second strongest factor in the logistic regression models, exceeded only by race. In models stratified by race, bone Pb was exceeded as a risk factor only by single parent status. The authors concluded that elevated body Pb burdens were associated with elevated risk for adjudicated delinquency.

The extension of Pb effects into delinquent and criminal behavior is significant for both the individual and society as a whole. Specific biological mechanisms that may underlie Pb effects on aggression, impulsivity, and poor self-regulation are not clearly understood. Lead impacts a large number of sites and processes in the brain involved in impulse control (Lidsky and Schneider, 2003). However, Needleman et al. (2002) proposed another pathway.
In addition to direct impacts on brain development and neuronal function, Pb exposure may increase risk of delinquency through a separate, indirect route: impaired cognitive abilities and

academic performance. That is, students who have difficulties in school and fail to achieve academic goals are more likely to become lawbreakers.

6.2.7 Sensory Acuities

In comparison to cognitive outcomes, there has been relatively less interest in Pb effects on sensory functions. However, there are clear indications that Pb exposure during the developmental period has an impact on complex aspects of visual and auditory acuities. Much of this work has been carried out in animal models (Otto and Fox, 1993). Epidemiologic studies have typically assessed hearing thresholds and features of auditory processing in Pb-exposed children. Studies reviewed in this section are summarized in Annex Table AX6-2.7.

Schwartz and Otto (1987) observed significant Pb-associated elevations in pure-tone hearing thresholds at various frequencies within the range of human speech among over 4,500 4 to 19-year-old subjects in NHANES II. In a later study, this finding was replicated in a sample of over 3,000 subjects aged 6 to 19 years in the Hispanic Health and Nutrition Examination Survey (HHANES) (Schwartz and Otto, 1991). An increase in blood Pb from 6 to 18 µg/dL was associated with a 2 db loss in hearing at all frequencies, and an additional 15% of children had hearing thresholds that were below the standard at 2,000 Hz. These relationships continued at blood Pb levels below 10 µg/dL.

Dietrich et al. (1992) assessed the relationship between scores on a test of central auditory processing (SCAN) and prenatal/postnatal blood Pb concentrations in 215 children 5 years of age drawn from the Cincinnati Lead Study. Higher prenatal, neonatal, and postnatal (up to concurrent) blood Pb concentrations were associated with more incorrect identification of common monosyllabic words presented under conditions of filtering (muffling). Other variables associated with impaired central auditory processing included results for pure-tone audiometry testing, social class, HOME scores, birth weight, gestational age, a measure of obstetrical complications, and consumption of alcohol during pregnancy. Following adjustment for these covariates, neonatal and postnatal blood Pb levels remained significantly associated with impaired performance on the Filtered Word subtest, more prominently in the right ear. In the right ear, the Filtered Word subtest score decreased by 0.7 points ($p < 0.05$; 95% CI not presented) for a 10 µg/dL increase in lifetime average blood Pb levels.

Osman et al. (1999) examined the relationship between concurrent blood Pb levels and hearing loss in 155 children 4 to 14 years of age living in an industrial region of Poland. Blood Pb levels ranged from 1.9 to 28 µg/dL (median 7.2 µg/dL). Hearing thresholds increased significantly with higher blood Pb levels at all frequencies (500-8,000 Hz). This relationship remained statistically significant when restricted to children with blood Pb levels <10 µg/dL.

A limited number of epidemiologic studies provide supportive evidence of a relationship between Pb exposure and auditory processing decrements. Lead-related deficits in hearing and auditory processing may be one plausible mechanism by which an increased Pb burden might impede a child's learning (Bellinger, 1995).

6.2.8 Neuromotor Function

Relatively few studies have focused on neuromotor deficits as an outcome of early Pb exposure. However, those that have examined motor functions in Pb-exposed children often report positive findings. Studies reviewed here are summarized in Annex Table AX6-2.8.

In an early study, unsteadiness, clumsiness, and fine-motor dysfunctions were noted in a group of mildly symptomatic Pb-poisoned children in Boston, with such effects persisting long after medical treatment (Pueschel et al., 1972). A study of moderately exposed children living in the vicinity of a longstanding Pb smelter in Greece found that children with blood Pb levels of 35 to 60 µg/dL had significantly lower scores on both the Gross and Fine Motor Composite scores from the Oseretsky scales when compared to control subjects (Benetou-Marantidou et al., 1988).

Only two modern prospective studies of Pb have assessed motor development in a comprehensive manner. Dietrich et al. (1993b) investigated possible Pb exposure associations with motor developmental status in 245 children 6 years of age in the Cincinnati Lead Study cohort. Following covariate adjustment, they found that postnatal Pb exposure was significantly associated with poorer scores on measures of bilateral coordination, visual-motor control, upper-limb speed and dexterity, and the fine motor composite from the Bruininks-Oseretsky scales. Neonatal, but not prenatal, blood Pb concentrations also were significantly associated with poorer scores on upper-limb speed and dexterity and the fine motor composite. The strongest and most consistent relationships were observed with concurrent blood Pb levels (mean 10.1 µg/dL [SD 5.6]). A 10 µg/dL increase in concurrent blood Pb levels was associated with

a 4.6 point (95% CI: 2.1, 7.1) decline in the fine motor composite score. In the same Cincinnati cohort, postnatal Pb exposure was associated with greater postural instability as assessed by a microprocessor-based strain gauge platform system (Bhattacharya et al., 1995). When assessed at 16 years of age, 78-month postnatal blood Pb levels were significantly associated with poorer fine-motor skills as indexed by covariate-adjusted factor scores derived from a factor analysis of a comprehensive neuropsychological battery (Ris et al., 2004). The variables loading highly on the fine-motor component came from the grooved pegboard and finger tapping tasks.

Some results of the Cincinnati Lead Study were replicated by Wasserman et al. (2000a) in the Yugoslavian Prospective Study. The Bruininks-Oseretsky Test of Motor Proficiency was adapted for use in their population residing in two towns in Kosovo Province. The main measure of exposure was the log of the lifetime average blood Pb concentration through 54 months of age. After covariate-adjustment, average childhood blood Pb levels were associated with poorer fine motor and visual motor function, but were unrelated to gross motor function.

A recent study by Després et al. (2005) of multiple exposures including Pb, Hg, and polychlorinated biphenyls (PCB's) found that only blood Pb levels measured at the time of assessment were associated with neuromotor functions in 110 preschool Inuit children residing in Canada. The mean blood Pb level was 5.0 µg/dL (range 0.8-27.1 µg/dL). Blood Pb levels were significantly associated with increased reaction time, sway oscillations, alternating arm movements, and action tremor. Only 10% of the children had blood Pb levels >10 µg/dL. After eliminating these children from the analyses, results remained significant for reaction time, sway oscillations, and alternating arm movements. These findings indicated that neuromotor effects of Pb occurred at blood Pb concentrations <10 µg/dL.

6.2.9 Brain Anatomical Development and Activity

Electrophysiological evaluations have been conducted on Pb-exposed children in order to obtain a more direct measure of the toxicant's impact on the nervous system. Studies reviewed in this section are summarized in Annex Table AX6-2.9. Much of this work was conducted by Otto and colleagues during the 1980s (e.g., Otto et al., 1985). These studies have demonstrated Pb effects on neurosensory functioning (auditory and visual evoked potentials) within a broad range of exposures (Otto and Fox, 1993).

Rothenberg et al. (1994) reported that higher maternal blood Pb levels at 20 weeks of pregnancy were associated with increased I-V and III-V interpeak intervals in the brainstem auditory evoked response recorded in 1-month-old infants. Mean maternal blood Pb level at 20 weeks in this subsample from the Mexico City Prospective Study was only 7.7 µg/dL, with a range of 1 to 30.5 µg/dL. Rothenberg et al. (2000) repeated these measurements with a larger group of 5- to 7-year-old children (n = 133). In contrast to their previous findings, prenatal blood Pb levels at 20 weeks were associated with decreased interpeak intervals. However, after fitting a nonlinear model to their data, they observed that I-V and III-V interpeak intervals decreased as blood Pb rose from 1 to 8 µg/dL and increased as blood Pb rose from 8 to 30 µg/dL. The biphasic effect was only observed with maternal blood Pb levels at 20 weeks of pregnancy. Increasing postnatal blood Pb at 12 and 48 months was related to decreased conduction intervals for I-V and III-V interpeak intervals across the entire blood Pb range.

Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) have recently been applied in studies of Pb-exposed children. Trope et al. (1998) were the first to apply MRI and MRS in an evaluation of a Pb-exposed subject (see Annex Table AX6-2.9 for a description of the case study). Trope et al. (2001) performed identical MRI and MRS studies on a sample of 16 subjects with a history of elevated blood Pb levels (23 to 65 µg/dL) before five years of age. Average age at time of evaluation was 8 years. These subjects were compared to age-matched controls composed of siblings or cousins who had blood Pb levels that never exceeded 10 µg/dL. Although all of the participants had normal MRI examinations, the Pb-exposed subjects exhibited a significant reduction in N-acetylaspartate: creatine and phosphocreatine ratios in frontal gray matter compared to controls.

Meng et al. (2005) performed MRI and MRS studies on children with blood Pb levels ≥27 µg/dL (n = 6) and age- and gender-matched controls with blood Pb levels <10 µg/dL (n = 6). The average age at time of evaluation was approximately 11 years. Subjects came from the Anhui province in China. Lead-exposed children had an average blood Pb concentration of 37.7 µg/dL (SD 5.7), while controls averaged 5.4 µg/dL (SD 1.5). MRS was used to measure N-acetylaspartate, choline-containing compounds, and total creatine in the frontal lobes and hippocampus in cases and controls. All children presented with normal MRI with no evidence of structural abnormalities. However, peak values of N-acetylaspartate, choline, and creatine in all four brain regions were reduced in Pb-exposed children relative to controls. The authors

concluded that the reduced brain N-acetylaspartate levels they observed in cases may be related to decreased neuronal density or neuronal loss. Furthermore, reduced choline signal may indicate decreased cell membrane turnover or myelin alterations that can lead to central nervous system hypertrophy, while lower creatine may indicate reduced neuronal cell viability.

Using functional MRI (fMRI), the influence of childhood Pb exposure on language function was examined in a subsample of 48 young adults from the Cincinnati Lead Study (Cecil et al. 2005; Yuan et al., 2006). At age 20-23 years, subjects performed an integrated verb generation/finger tapping paradigm. Higher childhood average blood Pb levels were significantly associated with reduced activation in Broca's area, a recognized region of speech production in the left hemisphere. This association remained statistically significant after adjustment for the subject's latest IQ assessment. Higher childhood blood Pb levels were also associated with increased activation in the right temporal lobe, the homologue of Wernicke's area (an area associated with speech production) in the left hemisphere. These results suggest that elevated childhood Pb exposure strongly influences neural substrates of semantic language function in normal language areas, with concomitant recruitment of contra-lateral regions resulting in a striking, dose-dependent atypical organization of language function.

6.2.10 Gene-Environment Interactions in the Expression of Lead-associated Neurodevelopmental Deficits

The discussion of gene-environment interactions with respect to Pb exposure encompasses differential susceptibilities with respect to race, gender, and genetic polymorphisms associated with Pb biodistribution, and neurotransmitter metabolisms and function. While the differential effects of Pb on neurodevelopment have been studied to some extent in regard to race and gender, little work has been reported with respect to specific genetic polymorphisms.

In the United States, African-American children are at increased risk for elevated blood Pb levels compared to White children. For example, in the last two NHANES surveys, African-American children were found to have significantly higher blood Pb levels than Whites, even after adjusting for urban residential status and family income (Brody et al., 1994; Mahaffey et al., 1982). However, reliable differences with respect to Pb effects on neurodevelopmental morbidity as a function of race have not been reported with consistency.

Most surveys find that boys have higher blood Pb levels than girls. The data are less clear with respect to gender-related differences in Pb-associated neurodevelopmental morbidities. At various assessments from birth to adolescence, a greater male vulnerability has been noted in the Cincinnati Lead Study (e.g., Dietrich et al., 1987b; Ris et al., 2004). Data from a cross-sectional study in England showed that the Pb-IQ deficit association was more pronounced in boys at 6 years of age (Pocock et al., 1987). However, in a study of 764 children in Taiwan, it was found that the relationship between Pb exposure and IQ scores was substantially stronger in girls (Rabinowitz et al., 1991). In the Port Pirie cohort study, Pb effects on cognition were significantly stronger in girls at ages 2, 4, 7, and 11-13 years (Baghurst et al., 1992; McMichael et al., 1992; Tong et al., 2000).

At least two genetic polymorphisms have been identified that appear to influence the absorption, retention and toxicokinetics of Pb in humans (Onalaja and Claudio, 2000). The δ-aminolevulinic acid dehydratase (ALAD) gene has been the most studied but, as yet, the consequences of the different alleles for susceptibility to the neurodevelopmental consequences of Pb exposure are unclear. Individuals with the ALAD 1-2 or ALAD 2-2 polymorphism tend to have higher blood Pb levels than those with ALAD 1-1. ALAD2 could increase vulnerability by raising blood Pb levels or decrease it by maintaining Pb in a sequestered state in the bloodstream. Only one pediatric study has examined this directly. Bellinger et al. (1994a) found that subjects with the ALAD2 polymorphism tended to have lower dentin Pb levels than those with ALAD1. This is consistent with the concept that increased affinity of the ALAD2 polymorphism inhibits entry of Pb from the blood stream into other tissues. After adjustment for exposure level, Bellinger et al. (1994a) found that adolescents with the ALAD2 polymorphism performed better in the areas of attention and executive functioning assessed in their study when compared to subjects with the ALAD1 polymorphism. However, because there were only 5 subjects with the ALAD2 form, meaningful statistical comparisons could not be made.

The other gene that has been studied is the vitamin D receptor or VDR gene. This gene is involved in calcium absorption through the gut. Research on Pb workers has shown that variant VDR alleles modify Pb concentrations in bone and the rate of resorption and excretion of Pb over time (Schwartz et al., 2000a). Haynes et al. (2003) examined the relationship between the VDR Fok1 polymorphism and blood Pb concentrations in 275 children enrolled in the Rochester Longitudinal Study. It was hypothesized that children homozygous for the F allele—a marker

for increased calcium absorption—would have higher blood Pb concentrations than heterozygotes and children homozygous for the f allele, after adjusting for environmental sources of Pb (floor dust Pb). A statistically significant interaction was found between floor dust Pb loading and VDR Fok1 genotypes on blood Pb concentration, with the FF genotypes having the highest adjusted mean blood Pb concentrations at 2 years of age. Consistent with other reports, Haynes et al. (2003) also found that African-American children were significantly more likely to have the VDR FF genotype than were non-African American children. The ability of African-American children to have increased calcium absorption may partially explain the higher blood Pb concentrations observed in African-American children. Unfortunately, there have been no studies to indicate which, if any, of the VDR polymorphisms are associated with increased vulnerability to the neurodevelopmental toxicity of Pb.

6.2.11 Persistence of Lead-Related Neurodevelopmental Deficits Associated with Prenatal and Postnatal Exposure

Neurodevelopmental effects of Pb have been shown to persist into later childhood and adolescence when environmental conditions have not markedly changed (i.e., no reduction in Pb exposure). The ramifications of Pb effects on neurodevelopment depend not only on the extent of the initially observable effects in early childhood, but also on their enduring consequences for cognition, attainment, and behavior over the lifetime of the individual. Recent studies examining the persistence of Pb-related neurodevelopmental deficits are summarized in Annex Table AX6-2.10. Key studies are further discussed in this section.

Since 1990, several studies attempted to eliminate or at least reduce Pb-associated neurodevelopmental damage through nutritional and/or pharmacological interventions. Optimism that such interventions might be effective was raised by a New York study published in the early 1990s. Ruff et al. (1993) observed that among children 13 to 87 months old (with blood Pb levels of 25-55 μg/dL) who were given chelation with EDTA and therapeutic iron, those with the greatest decline in blood Pb levels had improved cognitive test scores, independent of whether they had been given iron or chelation therapy.

The Treatment of Lead-Exposed Children (TLC) study was originally designed to test the hypothesis that children with moderate blood Pb levels who were given an oral chelating drug (dimercaptosuccinic acid or "succimer") would have better scores than children given placebo on

a wide range of tests measuring cognition, neuropsychological functions, and behavior at 36 months of follow-up (Rogan et al., 2001). The TLC study enrolled 780 children from four clinical sites into a randomized, placebo-controlled, double-blind trial of up to three 26-day courses of treatment with succimer. Most children lived in deteriorating inner-city housing, with 77% of the subjects being African-American. Succimer was effective in lowering the blood Pb levels of subjects on active drug during the first 6 months of the trial. However, after 1 year, differences in the blood Pb levels of succimer and placebo groups had virtually disappeared. All data analyses were conducted on an intent-to-treat basis. At 36 months of follow-up, the mean IQ score on the WPPSI-R of children given active drug was 1 point lower than that of children administered placebo, and children given succimer evinced more behavioral problems as rated by the primary caregiver on the Conners Parent Rating Scale. Children given succimer scored marginally better on the Developmental Neuropsychological Assessment (NEPSY), a battery of tests designed to measure neuropsychological deficits that can interfere with learning. However, all of these differences were statistically nonsignificant.

Although results for the first wave of follow-up for TLC were consistently negative for drug effects on cognition and behavior, they were not necessarily conclusive. Lead may affect higher-level neurocognitive processes that are inaccessible, difficult to assess, or absent in the preschool age child. In older children, scores on psychometric measures are more precise and reliable, a wider and more differentiated range of abilities can be examined, and early academic performance and social functioning outside the home environment can be evaluated. Therefore, TLC followed the cohort into the first years of elementary education to determine whether these later emerging neurodevelopmental functions were spared the effects of Pb in treated children compared to placebo controls (Dietrich et al., 2004). While remaining within the limits of hypothesis driven inference, a comprehensive battery of tests were administered to TLC subjects at 7 and 7.5 years of age. These included assessments of cognition, learning, memory, global intellectual attainment, attention/executive functions, psychiatric status, behavioral and academic conduct, neurological functioning, and motor speed. However, treatment with succimer resulted in no benefit in cognitive, behavioral, neurological, and neuromotor endpoints. Indeed, children treated with succimer fared worse than children in the placebo group in several areas, including linear growth, hospitalized and outpatient injury events in the first 3 years of follow-up, and neuropsychological deficits as assessed by the Attention and Executive Functions core domain

score from the NEPSY. The authors concluded that these latest follow-up data confirmed their earlier finding of the TLC regimen of chelation therapy not being associated with neurodevelopmental benefits in children with blood Pb levels of 20 to 44 µg/dL. These results furthermore, emphasize the importance of undertaking environmental control measures so as to prevent Pb exposure in light of the apparent irreversibility of Pb-associated neurodevelopmental deficits.

Liu et al. (2002) used the TLC succimer trial data set (Rogan et al., 2001) to examine the question of reversibility. As reviewed above, intent-to-treat analyses revealed no benefits of chelation on neurodevelopmental indices beyond 6 months of treatment. Thus, the scores on the cognitive tests from the two treatment groups could be analyzed either within the treatment groups or as a whole. Data from 741 children were available for analyses. Mean blood Pb levels in TLC subjects were 26.2 µg/dL at baseline, 20.2 µg/dL at the 6-month follow up, and 12.2 µg/dL at the 36-month follow-up. Mean declines in blood Pb levels were 6.0 µg/dL from baseline to 6-month follow-up, 14.1 µg/dL from baseline to 36-month follow-up, and 8.0 µg/dL from 6- to 36-month follow-ups. Blood Pb levels declined more quickly in the first 6 months in the succimer group than in the placebo group, but the mean blood Pb levels were very similar at baseline and at the 36-month follow-up. Prior to examining changes in blood Pb levels in relationship to changes in cognitive test scores, it was verified that baseline and later blood Pb levels were indeed significantly associated with deficits on measures administered at specific points in the study after adjustment for sociohereditary factors surveyed in the study, including maternal IQ. Unlike in the New York study by Ruff et al. (1993), Liu et al. (2002) found no overall effect of changing blood Pb level on changes in cognitive test score from baseline to 6 months. However, during the follow-up from baseline to 36 months and from 6 to 36 months, falling blood Pb levels were significantly associated with increased cognitive test scores, but only because of an association in the placebo group. Cognitive test scores increased by 2 points overall and 4 points in the placebo group when blood Pb levels declined by 10 µg/dL from baseline to 36 months. There is a possibility that the succimer drug regimen blunted the beneficial effect. Due to the inconsistency in the results, the data do not provide strong supportive evidence that Pb-induced cognitive impairments are reversible.

In addition to pharmacological interventions, a few studies have attempted to remediate or prevent Pb-associated neurodevelopmental deficits through nutritional supplementation. Again,

recent studies attempting to reduce Pb absorption through mineral hypersupplementation have been disappointing (Sargent et al., 1999). However, to date, there has been only one controlled clinical trial involving Pb-exposed children where central nervous system outcomes have been the focus of study. Kordas et al. (2005) and Rico et al. (2006) conducted a double-blind nutritional supplementation trial among 602 first grade children in the city of Torreón in northern Mexico. The city is located near a metal foundry that has been a source of Pb contamination in the community. The average blood Pb concentration at baseline was 11.5 µg/dL (SD 6.1) and about half of the children had blood Pb levels >10 µg/dL. Subjects received 30 mg ferrous fumarate, 30 mg zinc oxide, both, or placebo daily for 6 months. In their first report, the principal outcome assessment used at baseline and at follow-up was the parent and teacher forms of the Conners Rating Scales, with no consistently significant treatment effects being found and the authors concluding that this regimen of supplementation did not result in improvements in ratings of behavior in Pb-exposed children over 6 months. In addition to behavior, the authors also assessed cognitive functioning with 11 tests of memory, attention, visual-spatial abilities, and learning. Again, no consistent or lasting differences in cognitive performance were found among treatment groups, confirming the earlier conclusion that nutritional supplementation alone is not effective in eliminating or reducing the impact of early Pb exposure on functional neurodevelopment.

Children's blood Pb levels generally decline after they peak somewhere around 2 years of age. However, the degree of decline is a function of a number of factors, including previously acquired Pb body burden and sources of continuing exposure. Some observational studies have examined the extent to which the rate of decline in blood Pb levels may be associated with improvements in neurocognitive status. Tong et al. (1998) assessed the reversibility of Pb cognitive effects in early childhood in the Port Pirie, Australia cohort study. A total of 375 children were followed to the age of 11 to 13 years. Average blood Pb levels decreased from 21.2 µg/dL at 2 years to 7.9 µg/dL at 11-13 years. However, scores on standardized measures of intellectual attainment administered at 2, 4, 7, and 11-13 years of age in children whose blood Pb levels declined the most were not significantly improved over those obtained by children with a more shallow decline in Pb body burden.

Collectively, these studies indicate that nutritional and pharmacological interventions, without an effort to reduce environmental exposure to Pb, are relatively ineffective in improving

neurological function in children. Primary prevention and preventing additional increases in blood Pb levels among children whose blood Pb levels are high remain the most effective means of dealing with Pb toxicity.

6.2.12 Periods of Enhanced Developmental Susceptibility to Central Nervous System Effects of Environmental Lead

It has been difficult to identify discrete periods of development when the fetus or child is particularly susceptible to Pb effects on neurodevelopment. When the prospective studies of Pb and child development were underway, it was hoped that this methodological approach would be revealing. However, these studies observed that age strongly predicted the period of peak exposure (around 18-27 months when there is maximum hand-to-mouth activity), making it difficult to distinguish whether greater neurotoxic effects resulted from increased Pb exposure or enhanced susceptibility at a particular age. Furthermore, children with the highest blood Pb levels tended to maintain their rank order relative to their lower exposed peers throughout these studies (e.g., Dietrich et al., 1993a; McMichael et al., 1988), limiting the degree to which investigators could identify any particular period of development as being critical.

From the perspective of human neurodevelopmental biology, one could argue that the first 3 years of life should represent a particularly vulnerable period. The maximal period of Pb ingestion coincides with the same period of time when major events are occurring in the development of the central nervous system, including some neurogenesis, rapid dendritic and axonal outgrowth, synaptogenesis, synaptic pruning, and programmed apoptosis (see Figure 6-3).

The belief that the first 3 years of life represents a critical window of vulnerability is evident in the Pb literature (Chen et al., 2005). Two major meta-analyses of the relationships between childhood Pb exposure and IQ focused primarily on the strength of the association between IQ at school age and blood Pb concentrations at 2 years of age or average blood Pb levels up to 3 years of age (Pocock et al, 1994; Schwartz, 1994). Neither meta-analysis considered the importance of concurrent blood Pb associations in older children. The focus on these particular age groups implied that the interpretation most consistent with the overall results was that peak blood Pb concentration, achieved somewhere between 1 and 3 years of age, was most likely responsible for cognitive effects observed years later. These meta-analyses were highly influenced by findings from the Boston prospective study where blood Pb levels at

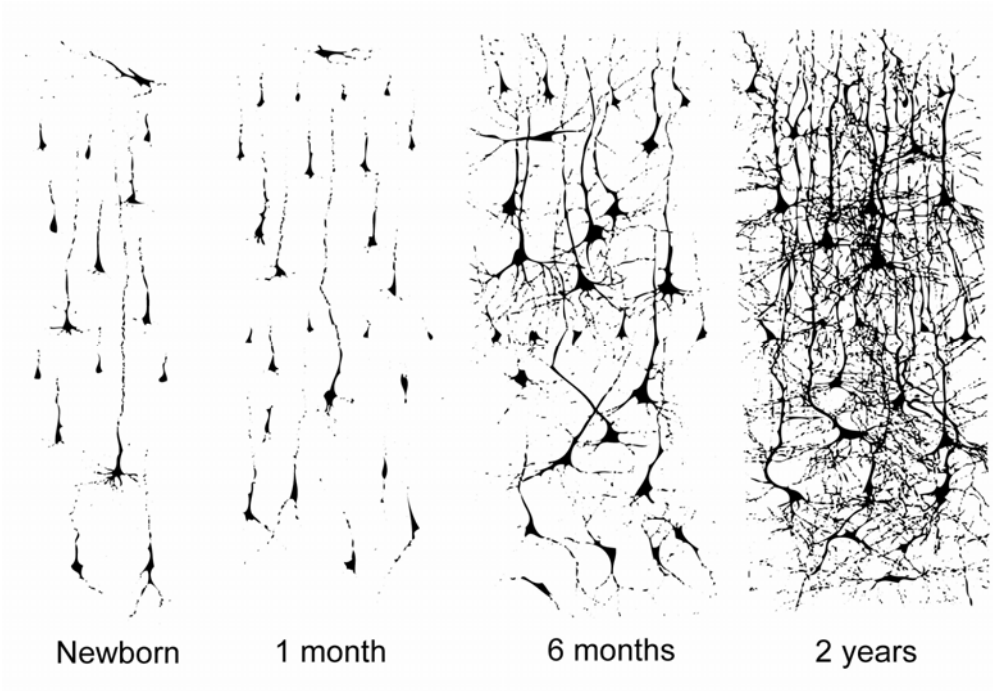

Figure 6-3. Golgi-stained section of human cerebral cortex taken from equivalent areas of the anterior portion of the middle frontal gyrus at different ages. Although the packing density of cortical neurons does not appear to change, there is a tremendous increase in the complexity of dendritic arborizations with increasing age with maximal density occurring between 2 and 3 years of age.

Source: Nolte (1993).

2 years of age have been exclusively and consistently associated with lower IQ and academic achievement (Bellinger et al., 1992).

This particular interpretation of the Pb literature has also influenced screening programs (which focus on 1 and 2 year olds), clinical trials that recruit children during the first 3 years of life, and current interpretation of the cross-sectional literature. For example, the report by Lanphear et al. (2000) that school-age children enrolled in the NHANES III survey displayed a significant inverse relationship between concurrent blood Pb concentrations and measures of IQ and academic achievement at blood Pb concentrations <10 µg/dL has been interpreted by some to reflect the effects of higher blood Pb concentrations in children when they were between 1 and 3 years of age.

Recent epidemiologic studies have found other blood Pb indices, including concurrent blood Pb levels or lifetime averages, to be stronger predictors of Pb-associated IQ effects than

peak blood Pb concentration. Prospective studies of children with both high and low Pb exposures found concurrent or lifetime average blood Pb levels to be more strongly associated with school age IQ and other measures of neurodevelopment (Canfield et al., 2003a; Dietrich et al., 1993a,b; Tong et al., 1996; Wasserman et al., 2000b). Lanphear et al. (2005) examined the relationship of IQ to four blood Pb indices — early childhood, peak, lifetime average, and concurrent blood Pb levels. All four blood measures were highly correlated, with correlation coefficients ranging from 0.74 to 0.96. The results of the regression analyses for the different blood Pb indices were very similar, but the concurrent blood Pb variable exhibited the strongest relationship with IQ as measured by R^2.

One recent study has attempted to directly address the question regarding periods of enhanced susceptibility to Pb effects. Chen et al. (2005) sought: to clarify the strength of the association between IQ and blood Pb at various time points, to examine whether cross-sectional associations observed in school age children 84-90 months of age represent residual effects from 2 years of age or "new" effects emerging among these children, and to evaluate how change in blood Pb over time is related to IQ at later ages. Chen et al. (2005) used data on 780 children from the previously-described TLC multicenter clinical trial (Dietrich et al., 2004; Rogan et al., 2001) to examine these relationships. Homogeneity between the two treatment groups was verified. There were no statistical differences between succimer and placebo groups in either blood Pb concentrations or cognitive scores at the time points under consideration. At baseline, children were given the Bayley Scales of Infant Development. The children's full scale IQ at the 36-month follow-up was measured with the WPPSI-R. At the 60 month follow-up, IQ was assessed with the WISC-III. All neurodevelopmental outcomes were adjusted for clinical center, race, gender, language, parent's education, parent's employment, single parent family, age at blood Pb concentration, and caregiver's IQ. Figure 6-4 displays the mean IQ at current and subsequent ages by quartiles of blood Pb measured at 2, 5, and 7 years of age. The concurrent blood Pb concentration always had the strongest association with IQ. As the children aged, the relationship grew stronger. The peak blood Pb level from baseline to 7 years of age was not associated with IQ at 7 years of age. Also, in models including both prior and concurrent blood Pb concentrations, concurrent blood Pb was always more predictive of IQ. Adjustment for prior IQ did not fundamentally change the strength of the association with concurrent blood Pb concentration. Chen et al. (2005) found a stronger relationship between IQ at 7 years of age and

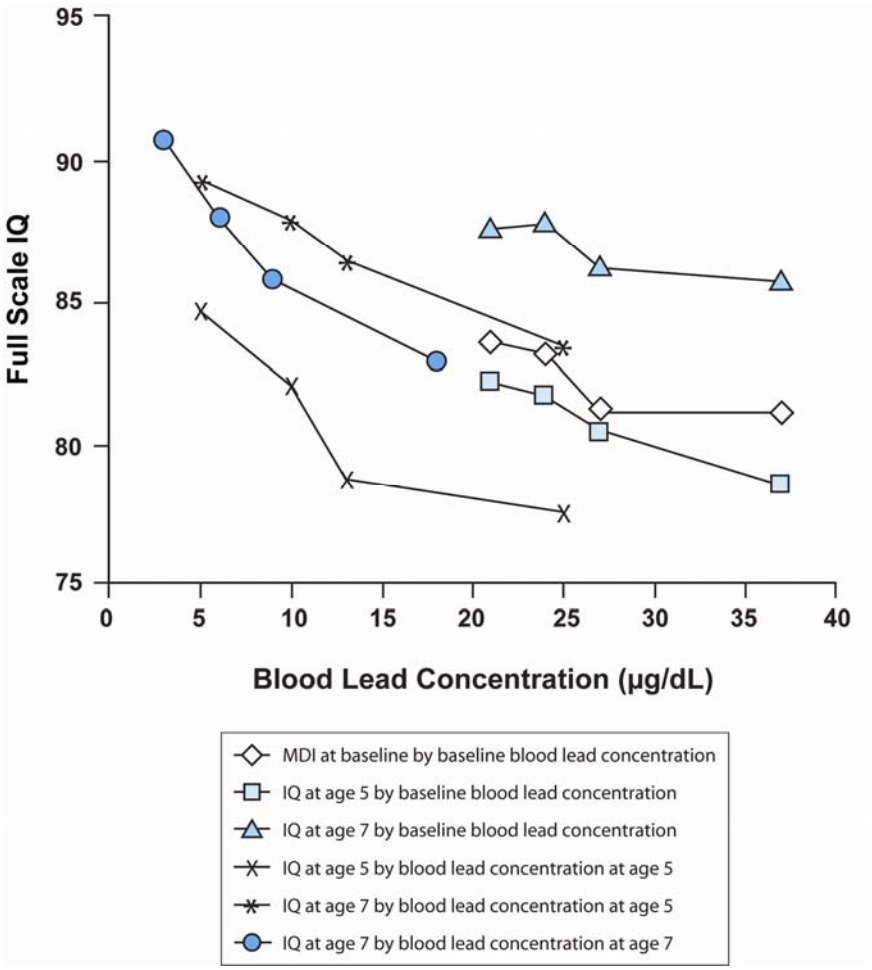

Figure 6-4. Full scale IQ test scores by previous or concurrent blood lead concentration. Each data point shows the mean IQ test scores of children measured at baseline or at two follow-up times, grouped by quartiles of blood lead concentration. The abscissa of each point is the middle value of each blood lead concentration category.

Source: Chen et al. (2005).

blood Pb concentration at 7 years compared with blood Pb at 2 years of age. A similar relationship was observed between IQ and blood Pb at 5 years of age. The strength of the cross-sectional associations increase over time, despite lower blood Pb levels in older children. These results support the idea that Pb exposure continues to be toxic to children as they reach school age, and do not lend support to the interpretation that all the damage is done by the time the child reaches 2 to 3 years of age. These findings also imply that cross-sectional associations seen in

children, such as the study recently conducted by Lanphear et al. (2000) using data from NHANES III, should not be dismissed. Chen et al. (2005) concluded that if concurrent blood Pb remains important until school age for optimum cognitive development, and if 6 and 7 year olds are as or more sensitive to Pb effects than 2 year olds, then the difficulties in preventing Pb exposure are magnified but the potential benefits of prevention are greater.

6.2.13 Effect of Environmental Lead Exposure on Neurodevelopment at the Lower Concentration Range

Over the past three decades, epidemiologic studies of Pb and child development have demonstrated inverse associations between blood Pb concentrations and children's IQ and other outcomes at successively lower Pb exposure levels. The 1986 Addendum and 1990 Supplement concluded that neurobehavioral effects were related to blood Pb levels of 10 to 15 µg/dL and possibly lower. In response to these data, agencies such as the U.S. CDC and the WHO have repeatedly lowered the definition of an elevated blood Pb concentration, which now stands at 10 µg/dL (CDC, 1991; WHO, 1995). At the time when these policies were put in place, there were too few studies of children with blood Pb levels consistently below 10 µg/dL on which to base an opinion as to effects at lower exposure levels. Since the removal of Pb from gasoline, the median blood Pb concentration has dropped dramatically in U.S. children, permitting more studies of this nature to be done in recent years. Also, the use of meta- and pooled analytic strategies has enabled investigators to get a clearer picture of effects below 10 µg/dL.

A recent review by the CDC of the epidemiologic literature on Pb effects on children's health concluded that the overall weight of available evidence supported an inverse association between blood Pb levels <10 µg/dL and the cognitive function of children (CDC, 2005). The CDC review further noted that a steeper slope in the dose-response curve appeared to be seen at lower compared to higher blood Pb levels. However, the CDC review also recognized several important limitations in the available evidence, most notably the small number of directly relevant cohort studies (i.e., studies that specifically examined the effect of blood Pb levels <10 µg/dL) and the inherent limitations of cross-sectional studies (i.e., the lack of data regarding blood Pb levels earlier in life). Since the CDC review, new publications have been added to the database evaluating the effect of blood Pb levels <10 µg/dL, which to some extent help address

the potential limitation of the number of relevant cohort studies. These are discussed in the following text.

Table 6-1 presents studies that examined the relationship between IQ and blood Pb level in children with blood Pb concentrations <10 μg/dL. The first group includes studies where all or the majority of the subjects in the study had blood Pb levels <10 μg/dL. The second group includes studies where an analysis has been done on the subset of children whose blood Pb levels were <10 μg/dL. In both groups, the average slope from the 10th percentile blood Pb level to 10 μg/dL was estimated. One additional study, Chiodo et al. (2004), examined the association between IQ and blood Pb levels in children with blood Pb levels <10 μg/dL. However, a slope estimate of change in full scale IQ per 1 μg/dL change in blood Pb could not be calculated from the standardized regression coefficients presented in the publication.

The Rochester Prospective Study (n = 172) by Canfield et al. (2003a) is illustrative. This study extended the relationship between deficits in IQ and blood Pb concentrations to levels well below 10 μg/dL. Over half of the children in this study did not have a recorded blood Pb concentration above 10 μg/dL. In covariate-adjusted linear models, each 1 μg/dL increase in concurrent blood Pb levels was associated with a −1.8 point decline in IQ at 5 years of age using only data from children with peak blood Pb levels <10 μg/dL compared to a −0.6 point decline in IQ when data from all children were included. Nonlinear semiparametric smoothing revealed a covariate-adjusted decline of more than 7 points up to 10 μg/dL of childhood average blood Pb, whereas a more gradual decline of ~2.5 points was associated with an increase in blood Pb from 10 to 20 μg/dL. In response to the Rochester findings, Bellinger and Needleman (2003) reanalyzed data from the Boston Prospective Study focusing on children whose blood Pb levels never exceeded 10 μg/dL (n = 48). In their analyses, 10 year IQ was inversely related to blood Pb levels at 24 months following adjustment for covariates. Nonparametric smoothing analyses indicated that the inverse association persisted at blood Pb levels below 5 μg/dL.

Other recent studies demonstrating effects below 10 μg/dL include a prospective study conducted in Mexico City by Téllez-Rojo et al. (2006). In a cohort of 294 children with blood Pb levels never exceeding 10 μg/dL, a statistically significant relationship was observed between blood Pb concentrations and MDI assessed concurrently at 24 months of age. Furthermore, a stronger effect of Pb on MDI was observed among infants with blood Pb levels <5 μg/dL.

Table 6-1. Summary of Studies with Quantitative Relationships of IQ and Blood Lead for Blood Lead Levels Less than 10 µg/dL

Reference	Study Location	n	Age of IQ Testing	Blood Lead Measurement	Model Used	Slope and 95% CI (IQ points/µg/dL) for Blood Lead <10 µg/dL[a]
Studies of Populations with Blood Leads <10 µg/dL						
Al-Saleh et al. (2001)[b]	Riyadh, Saudi Arabia	533	6-12 years	Concurrent	Log-linear	−0.8 (−1.4, −0.2)
Téllez-Rojo et al. (2006)	Mexico City, Mexico	294	24 months	Concurrent	Linear Log-linear	−1.0 (−1.8, −0.3) −0.9 (−1.4, −0.5)
Studies with Analyses Restricted to Subjects with Blood Leads <10 µg/dL						
Bellinger et al. (1992; reanalyzed Bellinger and Needleman, 2003)	Boston, Massachusetts	48	10 years	24 months[c]	Linear	−1.6 (−2.9, −0.2)
Canfield et al. (2003a)	Rochester, New York	101	5 years	Concurrent	Linear	−1.8 (−3.0, −0.6)
Kordas et al. (2006)	Torreón, Mexico	293	6-8 years	Concurrent	Linear	−0.4 (−1.2, 0.4)
Lanphear et al. (2005)[d]	International Pooled Analysis	244	4 years 10 months to 10 years	Concurrent	Linear Log-linear	−0.8 (−1.7, 0.1) −0.4 (−0.6, −0.3)

[a] The slopes for blood lead levels <10 µg/dL were estimated from the 10th percentile to 10 µg/dL.
[b] In Al-Saleh et al. (2001), 69% (n = 368) of the children had blood lead levels <10 µg/dL. The estimated slope is based on the model for the entire sample population.
[c] The original analyses by Bellinger et al. (1992) included slope estimates for concurrent as well as other blood lead measurements, including 24-month blood lead levels. Bellinger and Needleman (2003) presented reanalyses restricted to subjects with blood lead levels <10 µg/dL only for 24-month blood lead levels.
[d] The pooled analysis by Lanphear et al. (2005) included data from seven individual studies, including Bellinger et al. (1992) and Canfield et al. (2003a).

The most compelling evidence for effects at blood Pb levels <10 µg/dL comes from an international pooled analysis of seven prospective cohort studies (n = 1,333) by Lanphear et al. (2005) described earlier (Section 6.2.3.1.11). Although exposures in some cohorts were high, by pooling data from these studies a substantial number (n = 244) of children with blood Pb levels that never exceeded 10 µg/dL were included in the analyses.

The slope for Pb effects on IQ was steeper at lower blood Pb levels as indicated by the cubic spline function, the log-linear model, and the piece-wise linear model. Initially, the authors attempted to fit a linear model, but the shape of the dose-response relationship was determined to be nonlinear insofar as the quadratic and cubic terms for concurrent blood Pb were statistically significant ($p < 0.001$, $p = 0.003$, respectively). As illustrated in Figure 6-5, the shape of the spline function indicated that the steepest declines in IQ were at blood Pb concentrations below 10 µg/dL. Additional support for the notion of steeper slopes at lower blood Pb levels was given in Figure 6-2 (see Section 6.2.3.1.11), which presented the individual effect estimates for the seven studies used in the pooled analysis. The studies with the lowest mean blood Pb concentrations had a steeper slope compared with the studies with higher mean blood Pb concentrations.

The cubic spline is mainly used for descriptive purposes, but it can also be used to test for departure from linearity and from other nested models. It is not advisable to use the cubic spline as a concentration-response curve in a risk assessment because it is a complex model deliberately overfitting the data for the sole purpose of producing a smoothed representation of the trends in the data. The spline results agreed quite closely with the parametric log-linear model. Because the restrictive cubic spline indicated that a log-linear model provided a good fit of the data, the log of concurrent blood Pb was used in all subsequent analyses of the pooled data. Using a log-linear model, the authors estimated a decrement of 1.9 points (95% CI: 1.2, 2.6) in full scale IQ for a doubling of concurrent blood Pb. However, the IQ point decrements associated with an increase in blood Pb from <1 to 10 µg/dL compared to an increase from 10 to 20 µg/dL were 6.2 points (95% CI: 3.8, 8.6) versus 1.9 points (95% CI: 1.2, 2.6), respectively. Figure 6-6 illustrates the log-linear model and adjusted mean IQ for the intervals <5, 5-10, 10-15, 15-20, and >20 µg/dL. The vertical separation of the confidence limits on the log-linear regression line appear to be slightly wider at higher blood Pb levels. However, this separation increases symmetrically in both directions for log of blood Pb levels that are further away from the

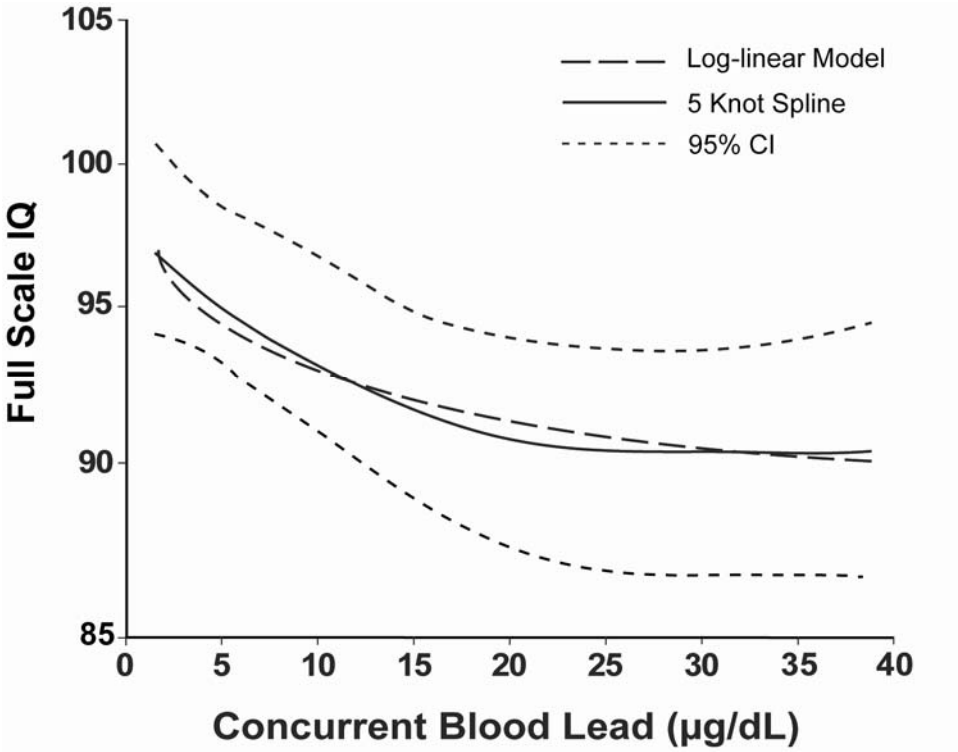

Figure 6-5. Restricted cubic splines and log-linear model for concurrent blood lead concentration. The dotted lines are the 95% confidence intervals for the restricted cubic splines.

Source: Lanphear et al. (2005).

geometric mean. The widening does not seem appreciable within the range of the observed blood Pb levels. The 5th and 95th percentile concurrent blood Pb values were 2.4 µg/dL and 33.1 µg/dL, respectively. The mean IQs for each of the specified intervals are within the confidence limits for the log linear model. These results support the conclusion that the observed steeper slopes at lower blood Pb levels in the pooled analysis were not due to the use of a log-linear model.

To further investigate whether the Pb-associated decrement was greater at lower blood Pb concentrations, the investigators divided the data at two cutpoints a priori, a maximal blood Pb of 7.5 and 10 µg/dL. Separate linear models were then fit to the data above and below the cutpoints and the concurrent blood Pb coefficients were compared (see Figure 6-7 for the analysis using the cutpoint of 10 µg/dL). The coefficient for the 103 children with maximal blood Pb levels

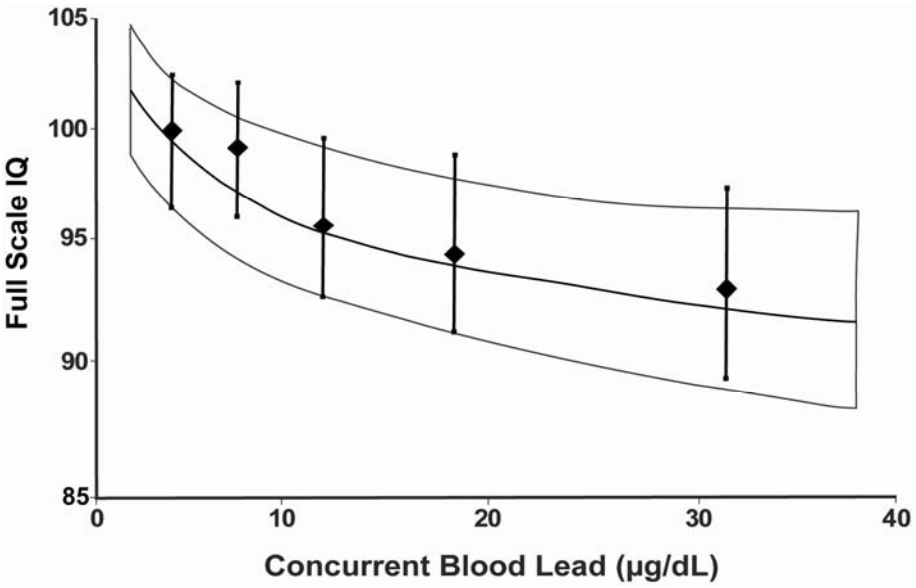

Figure 6-6. Log-linear model (95% CI shaded) for concurrent blood lead concentration adjusted for HOME score, maternal education, maternal IQ, and birth weight. The mean IQ (95% CI) for the intervals <5, 5-10, 10-15, 15-20, and >20 µg/dL are shown.

Source: Lanphear et al. (2005).

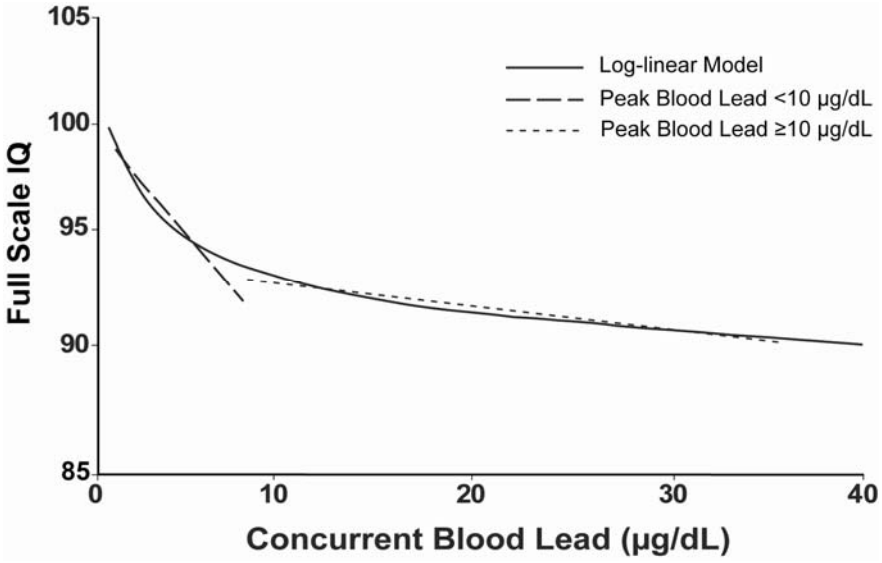

Figure 6-7. Log-linear model for concurrent blood lead concentration along with linear models for concurrent blood lead levels among children with peak blood lead levels above and below 10 µg/dL.

Source: Lanphear et al. (2005).

<7.5 μg/dL was significantly greater (p = 0.015) than the coefficient for the 1,230 children with a maximal blood Pb ≥7.5 μg/dL (−2.94 points [95% CI: −5.16, −0.71] per 1 μg/dL increase in blood Pb versus −0.16 points [95% CI: −0.24, −0.08]). The coefficient for the 244 children who had a maximal blood Pb <10 μg/dL also was greater than that for the 1,089 children who had a maximal blood Pb ≥10 μg/dL (−0.80 points [95% CI: −1.74, 0.14] versus −0.13 points [95% CI: −0.23, −0.03]), although the difference was not statistically significant (p = 0.10). Thus, while the pooled analysis used a log-linear model to quantify the Pb-associated decrements, the nonlinear relationship observed in the analysis was clearly not due to the influence of the log-linear model itself.

Rothenberg and Rothenberg (2005) reanalyzed the Lanphear et al. (2005) pooled study to examine the form of the concentration-response function for the Pb exposure effect on child IQ. This further analysis also focused on concurrent blood Pb levels. Rothenberg and Rothenberg reported that a log-linear relationship between blood Pb and IQ was a significantly better fit within the ranges of the blood Pb levels than was a linear relationship (p = 0.009), with little evidence of residual confounding from included model variables. Once again, this is consistent with a steeper slope at lower compared to higher levels of blood Pb.

For the entire pooled data set, the observed decline of 6.2 points in IQ for an increase in blood Pb from 1 to 10 μg/dL was comparable to the decrements for an increase in lifetime mean blood Pb levels from <1 to 10 μg/dL observed in the Rochester Longitudinal Study (Canfield et al., 2003a). The pooled analysis of Lanphear et al. also demonstrated that deficits in IQ extended to blood Pb levels <7.5 μg/dL. Therefore, recent evidence indicates that Pb is associated with neurocognitive deficits at blood Pb levels below 10 μg/dL in children. The data also suggest that there may be Pb effects associated with blood Pb <5 μg/dL, but the evidence is less definitive.

A common observation among some of these low blood-Pb level studies is that of nonlinear dose-response relationships between neurodevelopmental outcomes and blood Pb concentrations. At first this may seem at odds with certain fundamental toxicological concepts. However, there are a number of examples of nonlinear or supralinear dose-effect relationships in toxicology. It is conceivable that the initial neurodevelopmental lesions at lower Pb levels may be disrupting very different biological mechanisms than the more severe effects of high exposures that result in symptomatic poisoning or frank mental retardation (Dietrich et al., 2001). As Kordas et al. (2006) states, this might help explain why, within the range of exposures not

producing overt clinical effects, an increase in blood Pb beyond a certain concentration might cause less additional impairment in children's cognitive functions. It should be noted that the observation of nonlinear or supralinear dose-effect relationships between blood Pb and neurodevelopmental outcomes does not preclude the presence of a threshold, as blood or bone Pb levels currently measured in populations without obvious Pb poisoning are still orders of magnitude higher than those of pre-industrial humans (Patterson et al., 1991).

6.2.14 Selection and Validity of Neuropsychological Outcomes in Children

Considerable material has been written about methodologies for neurobehavioral evaluation in studies of environmental chemicals and child development (Bellinger, 2002, 2003; Dietrich et al., 2005). Much of the discussion has centered on the ability of neurobehavioral tests to detect damage to the central nervous system as a result of in utero or early postnatal Pb exposures. In other words, the sensitivity of these tests to toxicity has been in question.
The sensitivity of a neuropsychological or any other diagnostic test is defined as the proportion with the abnormality that the test classifies as abnormal (true positives). In the selecting of neurodevelopmental measures for use in studies of Pb or any other toxicant, it is clearly advantageous to include tests that have the best prognostic value. This is particularly important in the current context, because the neurobehavioral endpoints assessed in this document are being incorporated into an assessment of risk (Bellinger, 2002). In addition, it is important to select instruments that tap into neurodevelopmental domains that have been shown to be sensitive to particular environmental toxicants. As evident in this assessment, numerous neuropsychological instruments, tapping a wide range of domains have proven to be sensitive to lower level Pb exposure. Certain domains such as attention, executive functions, visual-spatial skills, fine-motor abilities, academic achievement (reading in particular), and externalizing behaviors appear to be affected by Pb with some degree of consistency. However, the identification of behavioral phenotypes for Pb has been a largely elusive goal. There are a number of plausible reasons for this. The sample's SES level, pattern and timing of Pb exposures; nutritional intake; general health; educational opportunities; and the particular instruments that were employed in a given study probably play an important role in contributing to between-study differences (Bellinger, 1995; Schantz, 1996). This may be one reason why the broad net provided by global, multiple domain assessments of cognition such as IQ have proven

to be the most consistently sensitive across studies of various design and sample characteristics. These measures combine subscales that are representative of a broad number of underlying cognitive functions; and they are thusly likely to pick up exposure-related deficits across cohorts that differ in their functional expressions of toxicity (Dietrich et al., 2005).

The validity of neuropsychological tests as indices of neurodevelopment in Pb studies is also of concern. In psychometrics, there are various types of validity. But the validity Pb researchers are usually most concerned about is "construct validity." If a measure has construct validity, it measures what it purports to measure. Most Pb researchers utilize assessments with proven construct validity. This means the instruments utilized by the investigator have been shown that they possess concurrent and predictive "criterion" validity (i.e., it relates to other manifestations of the construct that the instrument is supposed to be measuring and predicts an individual's performance in the future in specific abilities). It also means that the instrument possesses good "convergent validity." That is, that the test returns similar results to other tests that purport to measure the same or related constructs. Finally, the instrument should demonstrate "discriminant validity." This means that the instrument is not measuring a construct that it is not supposed to measure, but rather it discriminates.

Bellinger (2003) states that the general literature attests to robust observations between IQ and important measures of life success, such as grades in school, years of education, job success, social status, and income (Neisser et al., 1996; Salkever, 1995). Testing is difficult depending on examined age, especially for infants who are in a period of rapid developmental change. Also, the way an infant's cognitive function can be probed is restricted. The lack of continuity between their response modalities and ones that can be exploited as a child gets older is also a factor. Still neurobehavioral tests scores in infancy do possess strong concurrent validity.

There are many potential sources of invalidity which researchers take steps to avoid. These include unreliability (an instrument that, all other things being equal, yields scores that are unrepeatable and inconsistent) and bias (e.g., due to factors such as culture, gender). Most modern standardized measures of development and cognitive attainment have taken steps to reduce these sources of invalidity and must meet certain minimum requirements such as those formulated by the American Educational Research Association, American Psychological Association, and the National Council on Measurement in Education (American Educational Research Association et al., 1999). One reason that global measures of IQ have been used so

widely is because of their outstanding psychometric properties. The Wechsler series has excellent reliability and validity (Groth-Marnat, 2003). For example, the average internal consistency for the Wechsler children's scales across all age groups is 0.96. Test-retest reliability is similarly very high. The underlying factor structure of these scales has also been strongly confirmed. The validity of so-called experimental measures of learning and cognition is sometimes less certain.

All measurement procedures have the potential for error; so, the goal of the researcher is to minimize it. In elementary psychometric theory, any observed test score is made of the "true" score plus measurement error. It is assumed that the measurement error in the outcome variable is essentially random (the child's true score may not be reflected in the observed score because of errors of administration, inconsistency of administration across examiners, the child's health, or aspects of the testing environment that are not conducive to performance); thus, this measurement error does not bias the estimated effect size for exposure but does reduce the power to detect a significant effect. Therefore, efforts are made to minimize measurement error through attention to training, establishing inter-examiner reliability, attention to child factors, site factors, and vigilant monitoring of examiner performance throughout the course of a study (Dietrich et al., 2005).

6.2.15 Confounding, Causal Inference, and Effect Modification of the Neurotoxic Effects of Lead in Children

The major challenge to observational studies examining the impact of Pb on parameters of child development has been the assessment and control for confounding factors. By definition, a confounder is associated with both the exposure and the outcome and thusly has the potential to influence the association between the exposure and the outcome. Confounding by various factors can be controlled for in the design phase of the study or in the analytical phase. In the realm of Pb research, there are a wide range of potential confounders, the foremost of which is socioecomic status (SES). Socioeconomic status is measured rather crudely in most studies with such indices as the Hollingshead Four-Factor Index of Social Position that incorporates education and income of both parents. However, even these so-called blunt measures often account for a great deal of the variance in neurodevelopmental outcomes. Given the crude nature of these measures, to control for confounding by SES as well as rearing environment of the child,

many recent Pb studies have incorporated more direct assessments such as the HOME scale, parental intelligence, parental attitude assessments, and measures of parental substance abuse and psychopathology. Given the relatively high correlation between indices of Pb exposure and social environmental factors, the consistency among studies in finding effects following adjustment for these confounding factors is remarkable. In the Boston Prospective Study, confounding by SES was largely controlled for by study design (Bellinger et al., 1984). That is, the study subjects were generally middle- to upper-middle-class children in intact families with college-educated parents. Hence, the potential for confounding by SES in this study was considerably less compared to other Pb health effect studies; and yet it reported similar and, at times, even larger effects on neurodevelopmental outcomes. In addition, it is important to consider the extensive supporting experimental animal evidence not compromised by the possibility of confounding in examining Pb effects on health (Bellinger, 2004; Davis et al., 1990; U.S. Environmental Protection Agency, 1986a, 1990).

Another problem in the analyses of data regarding Pb effect on child development is the lack of critical consideration of which potential confounder in a particular model "owns" the variance in neurodevelopmental performance. Thus, for example, in the case of social class, it is assumed that if an effect of Pb is reduced to nonsignificance following adjustment for some measure of SES standing, the assumption is that all of the variance belongs to the confounder. However, in some instances this could be seen as an excessively conservative interpretation and raises the specter of Type II error. Social class could be seen as either a confounder or a proxy for exposure. In addition, Pb may be on the causal pathway of the association between social class and IQ. Lower social class in urban children is closely linked to residence in older housing in poor condition that, in turn, is associated with higher levels of environmental Pb (Clark et al., 1985). If studies adjust for social class in the usual manner, the effects of the toxicant will be underestimated (Bellinger, 2004). One extreme example of overcontrol of this nature can be found in the New Zealand studies where investigators regularly "controlled" for residence in older "weatherboard" housing (e.g., Fergusson et al., 1988a,b). However, it is worth noting that, even in the models including this variable, Pb remained a significant predictor of intellectual and academic under-attainment in the Christchurch Health Study. The proper way to address the possibility that Pb may be on the causal pathway of the association between social class and IQ is to use structural equation models, but this has not generally been done.

In addition to being a confounder, social class and related variables have been shown to be effect modifiers in many studies of Pb and child development (Bellinger, 2000; Tong et al., 2000). Effect modification occurs when the magnitude of an association between an exposure (Pb) and an outcome (neurobehavior) varies across strata of some other factor (Last, 2001). The disadvantages that accompany poor education and underemployment have been found to exacerbate Pb effects when carefully examined (Bellinger et al., 1989). Indeed, evaluating potential effect modifiers should be considered an important part of an overall data analytic plan.

Most of the important confounding factors in Pb studies have been identified, and efforts have been made to control them in studies conducted since the 1990 Supplement. Further discussion on confounding is presented in Section 6.10.3. Invocation of the poorly measured confounder as an explanation for positive findings is not substantiated in the database as a whole when evaluating the impact of Pb on the health of U.S. children (Needleman, 1995). Of course, it is often the case that following adjustment for factors such as social class, parental neurocognitive function, and child rearing environment using covariates such as parental education, income, and occupation, parental IQ, and HOME scores, the Pb coefficients are substantially reduced in size and statistical significance (Dietrich et al., 1991). This has sometimes led investigators to be quite cautious in interpreting their study results as being positive (Wasserman et al., 1997). This is a reasonable way of appraising any single study, and such extreme caution would certainly be warranted if forced to rely on a single study to confirm the Pb effects hypothesis. Fortunately, there exists a large database of high quality studies on which to base inferences regarding the relationship between Pb exposure and neurodevelopment. In addition, Pb has been extensively studied in animal models at doses that closely approximate the human situation. Experimental animal studies are not compromised by the possibility of confounding by such factors as social class and correlated environmental factors. The enormous experimental animal literature that proves that Pb at low levels causes neurobehavioral deficits and provides insights into mechanisms must be considered when drawing causal inferences (Bellinger, 2004; Davis et al., 1990; U.S. Environmental Protection Agency, 1986a, 1990).

6.2.16 Summary of the Epidemiologic Evidence for the Neurotoxic Effects of Lead in Children

Effects of Pb on neurobehavior have been reported with remarkable consistency across numerous studies of various designs, populations studied, and developmental assessment protocols. The negative impact of Pb on IQ and other neurobehavioral outcomes persist in most recent studies following adjustment for numerous confounding factors including social class, quality of caregiving, and parental intelligence. Moreover, these effects appear to persist into adolescence and young adulthood in the absence of marked reductions in environmental exposure to Pb.

- An international pooled analysis of seven prospective studies and several meta-analyses provide strong evidence that exposure to Pb at low dose has an effect on the intellectual attainment of preschool and school age children. Recent studies examining the Pb associations with intellectual attainment and academic performance in children with low Pb exposures have consistently observed effects at blood Pb concentrations below 10 µg/dL. The large international pooled analysis of 1,333 children estimated decline of 6.2 points (95% CI: 3.8, 8.6) in full scale IQ for an increase in concurrent blood Pb levels from 1 to 10 µg/dL.
- A common observation among some of these studies of low level Pb exposure is the nonlinear dose-response relationships between blood Pb and neurodevelopmental outcomes. At first this may seem at odds with certain fundamental toxicological concepts. However, a number of examples of non- or supralinear dose-response relationships exist in toxicology. It is conceivable that the initial neurodevelopmental lesions at lower Pb levels may be disrupting very different biological mechanisms (e.g., early developmental processes in the central nervous system) than the more severe effects of high exposures that result in symptomatic Pb poisoning and frank mental retardation.
- Studies examining aspects of academic achievement related to Pb exposure indicate the association of deficits in academic skills and performance, which in turn lead to enduring and important effects on objective parameters of success in real life.
- The effects of Pb on behavior and mood of children has been an important area of recent research. These studies have demonstrated that the impact of Pb may extend into increased risk for antisocial and delinquent behavior. This could be a consequence of attentional problems and academic underachievement among children who have suffered Pb exposures during their formative years.
- Several studies that have used methods of MRI and MRS to assess direct measures of brain damage are also adding important and direct evidence of harm due to Pb exposure. Reduced brain N-acetylaspartate levels observed may be related to decreased neuronal density or neuronal loss.

- It is not clear that only periods of peak blood Pb concentrations matter in terms of risks for neurodevelopmental morbidity. One study attempts to address this question directly and reports that concurrent blood Pb concentrations always had the strongest association with IQ as measured at ages 2, 5, and 7 years, with a stronger relationship as the children grow older.

- Attempts to reverse or limit Pb-associated neurodevelopmental morbidities with pharmacological or nutritional intervention strategies have thus far been ineffective. Epidemiologic studies are reporting effects at blood Pb levels for which there is no effective means of medical or secondary environmental interventions to avoid developmental morbidity, thus emphasizing the importance of taking primary protective measures to substantially reduce and ultimately prevent exposure of young infants and children to Pb.

6.3 NEUROTOXIC EFFECTS OF LEAD IN ADULTS

6.3.1 Summary of Key Findings on the Neurotoxic Effects of Lead in Adults from the 1986 Lead AQCD

Lead intoxication in adults occurred primarily in occupational settings with historically high Pb exposure levels. In more recent times, occupational Pb exposure has been reduced to much lower levels and is often associated with no symptoms. The symptom constellation associated with high levels of Pb exposure include impaired memory and attention span, irritability, headache, muscular tremors, and hallucinations (Cantarow and Trumper, 1944) that may progress to signs of frank encephalopathy (Smith et al., 1938). Symptoms of clinical Pb intoxication begin with blood Pb >40 μg/dL (Baker et al., 1979) accompanied by poorer performance on cognitive and visuomotor tasks, reaction time, verbal learning, and reasoning ability that reflect involvement of both the central nervous system and the peripheral nervous system (Arnvig et al., 1980; Campara et al., 1984; Grandjean et al., 1978; Haenninen et al., 1978, 1979; Hogstedt et al., 1983; Mantere et al., 1982; Valciukas et al., 1978; Zimmermann-Tansella et al., 1983). Impaired occulomotor function, measured by saccade accuracy and velocity, depended upon the age group of the Pb-exposed worker (Baloh et al., 1979; Glickman et al., 1984; Spivey et al., 1980).

With regard to peripheral nerve function as measured by nerve conduction studies, the 28 studies reviewed by the U.S. EPA in the 1986 Lead AQCD found no consistent single nerve

involved but, overall, the exposed group had slower conduction velocity at blood Pb levels as low as 30 μg/dL.

Studies reviewed in 1986 also found that amyotrophic lateral sclerosis (ALS) was inconsistently associated with elevated Pb levels in the nervous system. Chelation for 1 year did not did not alter elevated Pb levels in the tissue of patients with motor neuron disease.

6.3.2 Overview of Cognitive and Psychomotor Tests Used to Assess Adult Lead Exposure

Examination of Pb effects on neurobehavioral performance in adults differs from that in children, since the neurobehavioral tests in adults focus on loss of abilities previously present rather than the lack of attainment of those abilities. Also, there is the contribution of cognitive reserve acquired by years of education, self-education, on-the-job training, avocational, and non-avocational activities that increases the ability to compensate for the effects of Pb exposure on learning new information. However, cognitive reserve does not fully protect against the neurotoxic effects of Pb. The concept of brain reserve capacity has many examples in neurologic disease where neuropathology progresses in the absence of clinical expression—clinical parkinsonism develops once ~85% of nigrostriatal cells and dopamine are depleted; multi-infarct dementia is expressed once an aggregate volume of infarction involves 50 to 100 cc of the brain; the weakness and atrophy associated with poliomyelitis requires 80% loss of the anterior horn cells; and Alzheimer disease usually requires a frequency for senile plaques and neurofibrillary tangles of >60% in the hippocampus and cerebral cortex (Satz, 1993). Therefore, the goal is to identify these conditions in a preclinical phase. For instance, it is now known that, in individuals diagnosed with Mild Cognitive Impairment (only impairment of recent memory with preservation of other cognitive domains) are at increased risk of developing Alzheimer disease at rates of 12 to 15% per year compared to 1 to 2% in age-matched normal patients. Because of this brain reserve capacity and the decreased exposure to Pb both environmentally and occupationally, it is not expected to find clinical disease associated with exposure; however, subclinical effects, even if reversible, are important to identify as they may be impacting brain reserve capacity. It is known that diminished cognitive reserve increases the risk of decreased cognitive performance associated with Pb exposure (Bleecker et al., 2002). Therefore, at this time, it is more critical to study the contribution of Pb to performance after adjusting for

potential confounders and to identify subpopulations that are at increased risk for the neurobehavioral effects of Pb. A few studies have stratified outcome using clinical criteria and found higher Pb levels to be associated with more severe clinical abnormalities (Bleecker et al., 2003; Bleecker et al., 2005a).

Because alterations in mood may influence neuropsychological performance, many neurobehavioral batteries use self-administered questionnaires to screen for mood. For example, the Center for Epidemiologic Studies Depression Scale (CES-D) screens for depression. Also, the Profile of Mood State (POMS) screens for six subscales, namely anger, confusion, depression, fatigue, anxiety/tension, and vigor. The six mood scales of the POMS were originally validated in a clinical psychiatric population; thus, the factor structure needed to be validated in an occupational population. Factor analysis of the POMS in Pb smelter workers found only two relevant factors: one composed of five scales (anger, confusion, depression, fatigue, and tension) and the other contained vigor (Lindgren et al., 1999). This brings into question the use of the six scales as separate outcome variables in the study of Pb exposure.

The Mini-Mental-State Examination (MMSE), a screening tool for cognitive impairment, is a compilation of many cognitive domains, including orientation to time and place, registration, and recall of three words, attention, language, and visual construction, with a total possible score of 30 (Folstein et al., 1975). The MMSE is sensitive to age and education. In 194 healthy subjects aged 40 to 89 years with 7 to 21 years of education, only 1% of the subjects obtained an MMSE score of 24/30 and none below (Bleecker et al., 1988). MMSE errors are sensitive to age effects, including delayed recall, spelling "WORLD" backwards, and repetition of "no ifs, ands, or buts." With Pb exposure, examination of errors is important to compare with age-related changes and to determine the biological plausibility of the effects of exposure, especially when performing repeated measures of the test. This test is sometimes used to describe a population and not as an outcome.

Neuropsychological batteries used to screen for Pb effects in adults usually include the following domains (for a more complete description, see Lezak et al., 2004): attention/ concentration (Digit Span); conceptual and executive functioning (Stroop, Trails B); visuoperceptive/visuoconstructive (Block Design); visuomotoric (Reaction Time, Pegboard Test, Digit Symbol Substitution, Trails A); verbal memory (Rey Auditory Verbal Learning Test, Logical Memory, Paired Associated Learning); and nonverbal memory (Rey-Osterreith Complex

Figure, Benton Visual Retention). When analyzing possible associations between Pb exposure and test performance, adjusting for potential confounders is critical. Potential confounders are age, education (preferably a measure of verbal intelligence), depressive symptoms, medications, alcohol use, and smoking. In some cases, age and education may serve as effect modifiers; for example the association of Pb and poorer neurobehavioral outcome was greater in older workers (Bleecker et al., 1997a) or in those with less cognitive reserve (Bleecker et al., 2002).

Adults with medical conditions requiring medications that have nervous system side effects, history of severe head trauma, neurodegenerative disease and other neuropsychiatric conditions that may have a global impact on nervous system performance should be omitted from analyses for Pb effects.

6.3.3 Adult Environmental Lead Exposure Effects

6.3.3.1 Neurobehavioral Effects Associated with Environmental Lead Exposure

Exposure to chronic low levels of environmental Pb and its association with effects on the nervous system were examined in several populations originally followed to study conditions associated with aging: the VA Normative Aging Study (NAS) (Payton et al., 1998; Rhodes et al., 2003; Weisskopf et al., 2004a; Wright et al., 2003); the Study of Osteoporotic Fractures (Muldoon et al., 1996); the Kungsholmen Project on aging and dementia (Nordberg et al., 2000) and the third National Health and Nutrition Evaluation Survey, NHANES III (Krieg et al., 2005). Studies reviewed in this section are summarized in Annex Table AX6-3.1.

The VA Normative Aging Study (NAS) conducted at the VA Outpatient Clinic in Boston, MA is a multidisciplinary longitudinal investigation of the aging process established in 1961 and involving 2,280 men aged 21 to 80 years with no current or past chronic medical conditions. Participants are evaluated every three years with self-administered questionnaires and Brief Symptom Inventory (BSI) for psychiatric symptoms. By evaluating relationships of bone Pb (tibia 21.9 μg/g and patella 32.1 μg/g) and blood Pb (6.3 μg/dL) to psychiatric symptoms in 526 men (age 67 years), Rhodes et al. (2003) found education and mood symptoms for anxiety, depression, and phobic anxiety potentially to be associated with bone Pb levels after adjusting for age, age^2, alcohol, education and employment variables.

Neuropsychological testing in NAS found response speed to be sensitive to low levels of Pb, but it was not a consistent finding in all tests measuring the same domain upon examination

of 141 healthy men with a mean age of 67 years and 14 years of education. The mean blood Pb level was 6 µg/dL, patella bone Pb was 32 µg/g bone mineral, and tibia bone Pb was 23 µg/g bone mineral (Payton et al., 1998). Education was negatively correlated with bone and blood Pb levels. The handling of multiple comparisons was not addressed.

Another analysis of the NAS (Wright et al., 2003) examined 736 men, mean age 68 years with education level of 54% high school or less. The mean blood Pb was 5 µg/dL, and mean patella and tibia Pb levels were 30 and 22 µg/g bone mineral, respectively. The subjects had a mean MMSE score of 27. Relation of MMSE scores <24 (n = 41) and blood Pb by logistic regression estimated an odds ratio of 1.21 (95% CI: 1.07, 1.36). For patella and tibia Pb, odds ratios of 1.21 (95% CI: 1.00, 1.03) and 1.02 (95% CI: 1.00, 1.04), respectively, were observed. Risk of MMSE <24 (6% of the present population versus 1% of previously described healthy aging study), when comparing the lowest and highest quartiles, was 2.1 (95% CI: 1.1, 4.1) for patella Pb, 2.2 (95% CI: 1.1, 3.8) for tibia Pb, and 3.4 (95% CI: 1.6, 7.2) for blood Pb. Interaction of age with patella Pb and blood Pb in predicting MMSE found steeper decreases in MMSE scores relative to age in the higher quartiles of patella and blood Pb. Types of errors on the MMSE were not included. Not addressed was the issue of how medical conditions and medications that developed over the duration of the study were handled. Another publication on this population found that blue-collar participants in NAS had significantly more high school graduates as well as higher blood and bone Pb compared to white-collar participants, with non-White blue-collar workers having the highest bone Pb levels (Elmarsafawy et al., 2002).

Weisskopf et al. (2004a) expanded the MMSE study in NAS by examining 466 men (mean age 70 years), who had completed the MMSE twice with an interval of about 3.5 years. Mean blood Pb was 4 µg/dL, and mean patella and tibia bone Pb were 23 and 19 µg/g bone mineral, respectively. A one-interquartile range (20 µg/g bone mineral) higher patella Pb concentration was associated with a MMSE score change of −0.24. This association between patella Pb and change in MMSE score had a steeper inverse association at lower Pb levels. Baseline mean MMSE score was 27 and mean change in MMSE score of −0.24 was equivalent to aging 5 years on baseline MMSE scope. Five years of aging in a healthy population is not associated with any change in MMSE score (Bleecker et al., 1988). To address the biological plausibility of change in the MMSE over 3.5 years, errors by functional domain need to be identified to rule out the possibility of random errors with repeat performance.

Muldoon et al. (1996) studied participants in the Study of Osteoporotic Fractures for any association between nonoccupational Pb exposure and cognitive function. The Study of Osteoporotic Fractures began in 1986 and included women over age 65 years living in four different communities – Baltimore, MD; Portland, OR; Minneapolis, MN; and the Monongahela Valley outside of Pittsburgh, PA. A sample of 325 women from rural sites with a mean age of 71 years (mean blood Pb 4.5 µg/dL) and 205 women from urban sites with a mean age of 69 years (mean blood Pb 5.4 µg/dL) were examined. The urban group was more educated and had higher use of cigarettes and alcohol. Performance examined by blood Pb groups adjusting for age, education, smoking, and alcohol use found no significant differences in the urban group. However, in the rural group, individuals with blood Pb >7 µg/dL had significantly poorer performance when compared to those with blood Pb <4 µg/dL for Trails B, Digit Symbol, and Reaction Time. Response time across blood Pb groups increased for the rural group and decreased or remained the same for the urban group. Mean MMSE for the whole population was 25, with poorer performance in the rural group. MMSE scores as low as 15 were reported to be compatible with significant cognitive deterioration, as seen in Alzheimer's disease. Even though the neuropsychological battery was simple, 9 participants were unable to perform some of the tests including 3 on the MMSE. Such severe impairments were not found among those with higher occupational Pb exposures.

In the Kungsholmen Project on aging and dementia in Stockholm, Sweden, no relationship was found between blood Pb and MMSE (Nordberg et al., 2000). The study population included 762 participants, with a mean age of 88 years. The mean blood Pb in this group was 3.7 µg/dL, and the mean MMSE was 25. In contrast to the other populations examined, this study cohort was more homogenous, comprised entirely of elderly Swedes. Their likelihood of prior exposure to elevated Pb levels was low.

NHANES III administered 3 computerized neurobehavioral tests (simple reaction time, symbol-digit substitution, and serial digit learning) to 5,662 adults aged 20 to 59 years with a mean blood Pb of 3.30 µg/dL (range 0.7 to 41.8 µg/dL). No relationship between blood Pb and performance was found after adjusting for sex, age, education, family income, race/ethnicity, computer or video game familiarity, alcohol use, test language, and survey phase. Eleven adults with blood Pb levels between 25 and 42 µg/dL were analyzed separately, but no statistically significant relationship was found after adjusting for the covariates (Krieg et al., 2005).

6.3.3.2 Summary of Adult Environmental Lead Exposure Effects

The evidence relating environmental Pb exposure with impaired cognitive performance in the elderly is somwhat mixed. That is, several studies with adequate power using tests sensitive to the effects of Pb found no association between cognitive performance and blood Pb levels. On the other hand, those studies that evaluated both blood and bone Pb generally found significant associations between neurocognitive deficits and bone Pb, but not blood Pb. This suggests that long-term cumulative exposure, more than current exposure, may contribute to neurotoxic effects in adults.

6.3.4 Adult Occupational Lead Exposure Effects

Occupational studies on neurotoxic effects of Pb are summarized in Annex Tables AX6-3.2 through AX6-3.8. Key conclusions from these studies are briefly presented in this section.

Several studies observed a greater likelihood of neurological symptoms such as difficulty with concentration, irritability, and muscle pain in workers with elevated blood Pb levels (mean blood Pb levels >25 µg/dL) (Lucchini et al., 2000; Maizlish et al., 1995). The study by Lucchini et al. (2000) suggested a threshold for neurological symptoms at a blood Pb of 12 µg/dL. However, other studies with higher blood Pb levels (mean blood Pb levels >30 µg/dL) found no associations with symptoms related to the nervous system (Chia et al., 1997; Österberg et al., 1997).

Schwartz et al. (2001a) estimated that performances on psychomotor, motor speed, and dexterity begin to show a decline at a blood Pb threshold of 18 µg/dL. Some studies with mean current blood Pb levels <30 µg/dL found no significant associations with neurobehavioral performance, whereas cumulative blood Pb indices reflecting past high exposure were found to be a predictor of poorer performance (Bleecker et al., 1997a, 2005a; Lindgren et al., 1996).

Recent occupational Pb exposure studies consistently found peripheral sensory nerve impairment as opposed to the classic motor neuropathy described historically with high Pb exposure. Sensory nerve conduction studies, most commonly of the median nerve, were related to long-term exposure, lifetime integrated blood index, and duration of exposure or Pb body burden (Chia et al., 1996a,b; Kovala et al., 1997; Yokoyama et al., 1998). A possible threshold

for this effect on the sensory nerves was observed at a blood Pb of 28 to 30 μg/dL (Bleecker et al., 2005b, Chuang et al., 2000).

Visual evoked potentials (VEPs) and brainstem auditory evoked potential (BAEPs) measure speed of conduction in the visual and auditory pathways. A detailed study by Abbate et al. (1995) found blood Pb to be associated with prolonged VEPs with a threshold effect at 17 to 20 μg/dL. Four studies examining BAEPs and Pb exposure consistently found prolonged interpeak latencies in the brainstem auditory pathway more strongly associated with cumulative or weighted average blood Pb levels (Bleecker et al., 2003; Discalzi et al., 1992, 1993; Holdstein et al., 1986).

Postural sway is a complex task that requires the integration of visual, vestibular, and peripheral sensory inputs, as well as motor output. Various blood Pb indices have been associated with postural sway (Chia et al., 1994a, 1996c; Dick et al., 1999; Ratzon et al., 2000; Yokoyama et al., 1997). A benchmark dose level (the 95% lower confidence limit of Pb concentration resulting in an increased probability of an abnormal endpoint) for postural sway was calculated to be a current blood Pb level of 14 μg/dL by Iwata et al. (2005).

Parasympathetic and sympathetic integrity was compromised in Pb-exposed workers beginning at blood Pb levels ≥20 μg/dL and possibly lower (Ishida et al., 1996; Niu et al., 2000; Teruya et al., 1991). Quantitative electroencephalographs found increased beta activity associated with a mean blood Pb level of 29 μg/dL (Niu et al., 2000).

Several other studies examined the neurotoxic effects of occupational exposure to organolead (e.g., trimethyl Pb, tetraethyl Pb) (Balbus et al., 1997, 1998; Schwartz et al., 1993, 2000b, 2001b; Stewart et al., 1999). Exposure to tetraethyl Pb was associated with poorer performance in many cognitive domains, but most often in manual dexterity and verbal memory/learning.

6.3.5 Amyotrophic Lateral Sclerosis and Other Neurological Outcomes Associated with Lead in Adults

Studies reviewed in this section are summarized in Annex Table AX6-3.9. The 1986 Lead AQCD concluded that the evidence for an association of Pb and ALS or motor neuron disease was inconsistent. The subsequent publications remain mixed, but more studies have reported an association. Using 109 cases of ALS and 256 controls matched for age, gender,

and region of residence, Kamel et al. (2002) examined the relation of Pb to ALS, using blood Pb and bone Pb levels. Ranges of exposure were <1 to 14 μg/dL for blood Pb, −4 to 107 μg/g for patella Pb, and −7 to 61 μg/g for tibia Pb. History of occupational Pb exposure increased the risk of ALS (adjusted odds ratio of 1.9 [95% CI: 1.1, 3.3]). Elevations both in blood Pb and in patella and tibia bone Pb were found in ALS cases, though the precision of these measurements was questioned. In summary, this study found Pb exposure from historical questionnaire data and biological markers to be associated with ALS. The same data were used to determine the associations of ALS with polymorphism in ALAD and VDR and the influence of genotype in the previously discussed associations of ALS with Pb (Kamel et al., 2003). The ALAD2 allele was associated with a 2-fold increased risk of ALS after adjustment for age, gender, region, education, and physical activity. Additionally adjusting for blood Pb strengthened the association of ALAD2 and ALS risk. This was not found for bone Pb or occupational history of Pb exposure. VDR was not associated with Pb or ALS risk.

A study from the Mayo Clinic examined risk factors for sporadic ALS in 45 male ALS patient-patient control pairs (Armon et al., 1991). When lifetime exposure to Pb exceeded 200 hours, the relative risk for ALS was 5.5 (95% CI: 1.44, 21.0). Overall, men with ALS had worked more at blue-collar jobs with significantly more time welding or soldering than controls ($p < 0.01$). The association between Pb exposure and development of ALS was supported, as these authors had the same findings in a previous pilot study of another patient population (Roelofs-Iverson et al., 1984).

Another study of risk factors for ALS in 103 patients found increased odds ratio for manual occupation (2.6 [95% CI: 1.1, 6.3]) and occupational exposure to Pb (5.7 [95% CI: 1.6, 30]) (Chancellor et al., 1993). A Swedish study of 92 cases of motor neuron disease (includes ALS, progressive bulbar palsy, and progressive muscular atrophy) found a Mantel-Haenszel odds ratio for welding equal to 3.7 (95% CI: 1.1, 13.0) (Gunnarsson et al., 1992).

Guidetti et al. (1996) performed a retrospective incidence, prevalence, and mortality survey in northern Italy. The area studied had documented Pb pollution for years. Based upon 79 cases, incidence and prevalence rates of ALS were comparable to the surrounding area. A subsequent publication by this group found that mean blood Pb levels in cases of sporadic ALS and controls were not significantly different (mean blood Pb of 13 μg/dL versus 11 μg/dL)

(Vinceti et al., 1997). Blood Pb was associated with disability due to ALS but no support was found for involvement of Pb in the etiology of sporadic ALS.

Louis et al. (2003) examined the relationship between blood Pb and essential tremor (ET) in 100 cases with ET (mean blood Pb 3 μg/dL) and 143 controls (mean blood Pb 2 μg/dL). Ten cases and 7 controls had bone Pb levels measured that were significantly correlated with blood Pb, suggesting that higher blood Pb may have occurred in the past. Logistic regression adjusting for age and current cigarette smoking found an association between blood Pb and ET. An odds ratio of 1.19 (95% CI: 1.03, 1.37) was estimated. Blood Pb was higher in the 39 ET cases with no family history. Both current and lifetime prevalence of occupational Pb exposure was the same in ET cases and controls. In a second publication (Louis et al., 2005), 63 ET cases (mean blood Pb of 4 μg/dL) and 101 controls (mean blood Pb of 3 μg/dL) who were similar in age, education, gender, and ethnicity were examined for interaction of blood Pb and ALAD gene polymorphisms and increased odds of ET. Of the 63 ET cases, 18 (29%) had an ALAD2 allele compared to 17 (17%) of the 101 controls (odds ratio of 1.98 [95% CI: 0.93, 4.21]). When log blood Pb was examined by presence of ALAD2 allele in ET, log blood Pb was highest in ET cases with the ALAD2 allele, intermediate in ET cases without an ALAD2 allele, and lowest in controls (test for trend, $\beta = 0.10$; $p = 0.001$). When the ALAD2 allele was present, blood Pb was significantly associated with odds of ET (80.29 [95% CI: 3.08, 2,096.36]). This increased odds of ET with an ALAD2 allele was 30 times greater than in individuals with only ALAD1 alleles. In the highest log blood Pb tertile, ALAD2 allele was present in 22% of ET cases and 5% of controls. It was proposed that increased blood Pb along with the ALAD2 allele could affect the cerebellum and, thereby, increase the risk of tremor.

Graves et al. (1991) performed a meta-analysis on 11 case-control studies of Alzheimer's disease for occupational exposure to solvents and Pb. Four studies had data for Pb exposure, with a pooled analysis of relative risks for occupational Pb of 0.71 (95% CI: 0.36, 1.41). The exposure frequencies were 16 of 261 (6%) for the cases and 28 of 337 (8%) for the controls. These nonsignificant results were further confirmed by measuring Pb concentration in the brain of cases with diffuse neurofibrillary tangles with calcification (DNTC), Alzheimer's disease, and non-demented controls. The Pb concentration was significantly higher in DNTC compared to Alzheimer's disease and non-demented controls (Haraguchi et al., 2001).

In summary, more studies are reporting an association with past exposure to Pb, usually in the occupational setting, and the motor neuron disease ALS. There appears to be a 2-fold increased risk for ALS when the ALAD2 allele is present. The odds of ET in individuals with the ALAD2 allele were 30 times greater compared to those with only ALAD1 alleles. No increased risk of Alzheimer's disease was related to Pb exposure.

6.3.6 Summary of the Epidemiologic Evidence for the Neurotoxic Effects of Lead in Adults

Neurobehavioral tests in adults focus on loss of abilities previously present. Cognitive reserve acquired by years of education and life activities increases the ability to compensate for the effects of Pb exposure on learning new information. However, it should be noted that cognitive reserve is not fully protective against the neurotoxic effects of Pb. Several new publications evaluate effects associated with environmental Pb exposure and other information is related to effects associated with occupational Pb exposure.

- In the limited literature examining environmental Pb exposure, there appears to exist mixed evidence regarding associations between Pb and impaired cognitive performance in adults. Studies using concurrent blood Pb levels as the marker for Pb exposure found no association between cognitive performance and Pb exposure. However, significant associations were observed in relation to bone Pb concentrations, suggesting that long-term cumulative exposure may be crucial in contributing to neurocognitive deficits in adults.
- Chronic occupational Pb exposure was found to be associated with peripheral sensory nerve impairment, visuomotor and memory impairment, prolonged VEPs and BAEPs, and postural sway abnormalities. A possible threshold at blood Pb levels ≥ 14 µg/dL was observed for these neurotoxic effects.
- Past occupational exposure to Pb increased the risk of developing ALS and motor neuron disease in 4 studies. This risk was increased 2-fold by the presence of the ALAD2 allele. Essential tremor in two well-done studies was associated with low blood Pb levels (mean 3 µg/dL). The odds of developing ET with the ALAD2 allele increased 30-fold compared to those individuals with only an ALAD1 allele.

6.4 RENAL EFFECTS OF LEAD

6.4.1 Summary of Key Findings on the Renal Effects of Lead from the 1986 Lead AQCD

Chronic Pb nephropathy is a disease characterized by tubulointerstitial nephritis, which can ultimately result in small, fibrotic kidneys. It occurs in individuals who sustain chronic high-level Pb exposure. In these individuals, Pb exposure is the primary cause of renal failure. The pathophysiologic characteristics of Pb nephropathy and the populations at increased risk for this diagnosis were the foci of the human research portion of Section 12.5, entitled "Effects of Lead on the Kidney," in the 1986 Lead AQCD. The 1986 document clearly identified several high-risk groups for this diagnosis, including children in the Queensland, Australia Pb poisoning epidemic, moonshine alcohol drinkers, and Pb workers in poorly controlled settings. The section concluded that data in the latter group indicated an increased risk for Pb nephropathy associated with blood Pb levels ranging from 40 to >100 μg/dL, with adverse renal effects possibly occurring at levels as low as 30 μg/dL.

The 1986 Lead AQCD noted that research at that time was not sufficient to address some of the most critical questions relating to the impact of Pb exposure on the kidney. The last paragraph of the renal section begins with "Among the questions remaining to be answered more definitively about the effects of Pb on the kidneys is the lowest blood Pb level at which renal effects occurs." The last sentence reads "Conversely, the most difficult question of all may well be to determine the contribution of low levels of Pb exposure to renal disease of non-Pb etiologies." Advances in the research conducted since that document was written allow a much more informed discussion of exactly those critical issues. As discussed below, recent research indicates that Pb nephropathy is merely the tip of the iceberg in terms of the contribution that Pb makes to renal dysfunction overall. Research increasingly indicates that Pb, at much lower doses than those causing Pb nephropathy, acts as a cofactor with other more established renal risks to increase the risk for renal dysfunction and the rate of subsequent decline. The populations at risk for renal dysfunction (diabetics and hypertensives) are increasing worldwide, particularly in countries where obesity is epidemic. Pb exposure is declining in many industrialized countries, although less so among high-risk minority populations. The extent of the public health impact of Pb on the kidney depends on the balance of these two factors.

6.4.2 Renal Outcome Definitions

The renal literature can be confusing, because several of the clinical renal measures are inversely related. Therefore, the pertinent outcomes are briefly reviewed below. The glomerular filtration rate (GFR) is considered to be the best measure of renal function. GFR is assessed by urinary clearance of exogenous (e.g., ^{125}I-iothalamate) or endogenous (e.g., blood urea nitrogen [BUN] and serum creatinine) compounds. Creatinine is used most commonly. Therefore, increases in BUN or serum creatinine or decreases in renal clearance of creatinine or other markers are all consistent with decreased renal function. Serum creatinine and its reciprocal have been the most frequently used measures of renal function in the Pb-kidney literature. However, creatinine is not an ideal GFR marker, because it is influenced by factors such as muscle mass, diet, gender, age, and tubular secretion. Measurement or calculation of creatinine clearance takes some of these variables into account. Measured creatinine clearance utilizes timed urine collections, traditionally over a 24-h period, making compliance difficult. Therefore, equations to estimate creatinine clearance have gained popularity. The Cockcroft-Gault equation (Cockcroft and Gault, 1976) has been used most commonly. Recently, several equations to estimate actual GFR were studied in the Modification of Diet in Renal Disease (MDRD) Study (Levey et al., 1999). The abbreviated MDRD equation (GFR in mL/min/1.73m^2 = 186 $\times$ creatinine$^{-1.154}$ $\times$ age$^{-0.203}$ $\times$ (0.742 if female) $\times$ (1.212 if African-American); Stevens and Levey [2005a]) estimates GFR more accurately than the Cockcroft-Gault equation in patients with renal insufficiency (Levey et al., 2003). Despite their promise, however, the MDRD equations are relatively new and their use in studies of renal effects of Pb exposure has been limited to date.

Cystatin C is another recent addition to the tools used to assess GFR (Stevens and Levey, 2005b). This is a 13,000 Dalton, non-glycosylated basic protein, which is generated by all nucleated cells and filtered, reabsorbed, and catabolized, but not secreted, in the kidney. Very little appears in the urine. The majority of studies done to date indicate that serum cystatin C is a better marker for GFR than serum creatinine (Stevens and Levey, 2005b).

Most of the renal outcome measures discussed above were developed for use in the clinical setting. Unfortunately, they are insensitive for early renal damage, as evidenced by the fact that serum creatinine remains normal after kidney donation. Therefore, in the last two decades, the utility of renal early biological effect (EBE) markers as indicators of preclinical renal damage has been of interest. These can be categorized as markers of function (i.e., low

molecular weight proteins that should be reabsorbed in the proximal tubules such as β_2-microglobulin and retinol-binding protein [RBP]); biochemical alteration (i.e., urinary eicosanoids such as prostaglandin E_2, prostaglandin $F_{2\ alpha}$, 6-keto-prostaglandin $F_{1\ alpha}$, and thromboxane B_2); and cytotoxicity (e.g., N-acetyl-β-D-glucosaminidase [NAG]) (Cárdenas et al., 1993). Elevated levels may indicate an increased risk for subsequent renal dysfunction. However, with the exception of microalbuminuria in diabetes and β_2-microglobulin in Cd exposure, most are research tools only, and their prognostic value remains controversial. Asian and European nephrotoxicant researchers have used them more frequently than have U.S. renal researchers. Prospective studies of most of these markers in nephrotoxicant-exposed populations are quite limited to date.

6.4.3 Lead Exposure Measure Definitions

Although these definitions are reviewed in detail elsewhere in this Lead AQCD, a brief discussion is included here due to the number of key studies in this section that measured bone or chelatable Pb dose. Inorganic Pb is a cumulative toxicant that is stored in bone. Blood Pb is a relatively short-term measure (half-life of 30 days [Hu et al., 1998]) that reflects exposure from current exogenous sources and the release of Pb from internal Pb stores. Bone is an internal source of Pb as well as a repository (Hu et al., 1998). As such, bone Pb measures provide an index not only of cumulative Pb exposure but also the potential for ongoing internal exposure, as well. Lead in trabecular bone (commonly measured in the patella or calcaneus) is more bioavailable than Pb in cortical bone (measured in the mid-tibia) and has a shorter half-life (Gerhardsson, et al., 1993; Hu et al., 1998). An additional Pb measure, chelatable Pb, is thought to represent a bioavailable pool of Pb from blood, soft tissue, and bone. Two chelation agents, either calcium disodium ethylenediaminetetraacetic acid (EDTA) or dimercaptosuccinic acid (DMSA; succimer) have mainly been used for this purpose, although DMSA is newer and, thus, used less frequently to date.

6.4.4 Lead Nephrotoxicity in Adults

6.4.4.1 General Population Studies

Over the past two decades, several studies have examined the effect of Pb exposure on renal function in general populations. This is a new category of Pb-renal research. No high

quality examples (by current standards) were available for review in the 1986 Lead AQCD. The studies discussed below provide critical evidence that the adverse effects of Pb on the kidney occur at much lower doses than previously appreciated. Traditional renal function measures, such as serum creatinine, BUN and creatinine clearance, are emphasized below, since much more is known regarding the clinical relevance of these measures than for the renal EBE markers. General population studies of the renal effects of Pb are further summarized in Annex Table AX6-4.1.

6.4.4.1.1 Cadmibel Study

In the first large environmental study that adjusted for multiple renal risk factors, Staessen et al. (1992) evaluated 965 men and 1,016 women in the Belgian Cadmibel study. Lead dose was indexed by blood Pb and zinc protoporphyrin. Renal outcome measures included (a) serum creatinine and β_2-microglobulin and (b) 24-h measured and calculated (Cockcroft and Gault, 1976) creatinine clearances. Mean blood Pb was 11.4 µg/dL (range 2.3-72.5) and 7.5 µg/dL (range 1.7-60.3) in men and women, respectively. After adjustment, log transformed blood Pb and zinc protoporphyrin, in separate models, were negatively associated with measured creatinine clearance. A 10-fold increase in blood Pb was associated with a decrease in creatinine clearance of 10 and 13 mL/min in men and women, respectively. Both Pb measures were also negatively associated with estimated creatinine clearance. This landmark study raised concern that the Pb dose threshold for adverse renal effects in the general population might be much lower than had been previously appreciated based on occupational exposure data.

6.4.4.1.2 Normative Aging Study

Research in the Normative Aging Study population reached similar conclusions. Four studies assessing the renal impact of Pb exposure in this population have thus far been published. Participants in this study were originally recruited in the 1960s in the Greater Boston area. Inclusion criteria included male gender, age 21 to 80 years, and absence of chronic medical conditions. Payton et al. (1994) analyzed data from a periodic follow-up evaluation performed between 1988 and 1991 in 744 participants. Lead dose was indexed by blood Pb; renal outcome measures included serum creatinine and 24-h measured and calculated (Cockcroft and Gault, 1976) creatinine clearances. Mean blood Pb concentration and measured creatinine clearance

were 8.1 μg/dL (SD 3.9) and 88.2 mL/min (SD 22.0), respectively. After adjustment, ln blood Pb was negatively associated with ln measured creatinine clearance ($\beta = -0.04$ [95% CI: −0.079, −0.001]). Borderline statistically significant associations ($p < 0.1$) between blood Pb and serum creatinine and estimated creatinine clearance were also observed. Kim et al. (1996) studied 459 men whose blood Pb levels from past periodic examinations, conducted every 3 to 5 years during 1979-1994, were measured from stored samples. Participants were randomly selected to be representative of the entire Normative Aging Study population in terms of age and follow-up. Renal status was assessed with serum creatinine. Data from 4 to 5 evaluations were available for the majority of participants. Relations were evaluated cross-sectionally (associations between blood Pb and concurrent serum creatinine) as well as longitudinally (associations between blood Pb and change in serum creatinine over the subsequent follow-up period). Mean age, blood Pb level, and serum creatinine, at baseline, were 56.9 years (SD 8.3), 9.9 μg/dL (SD 6.1), and 1.2 mg/dL (SD 0.2), respectively. With random-effects modeling, a significant positive association between ln-transformed blood Pb and concurrent serum creatinine was observed. This association was stronger when models were confined to participants with lower peak blood Pb levels, i.e., the β coefficient was largest in the 141 participants whose highest blood Pb level was ≤10 μg/dL ($\beta = 0.06$ [95% CI: 0.023, 0.097]). In the longitudinal analysis, ln-transformed blood Pb was associated with change in serum creatinine over the subsequent follow-up period in the 428 participants whose highest blood Pb level was ≤25 μg/dL ($\beta = 0.027$ [95% CI: 0.0, 0.054]). Similar to the cross-sectional analysis, the β coefficient in the participants whose highest blood Pb level was ≤10 μg/dL was larger; however, in the longitudinal analysis, the standard error also increased such that the p-value was not significant.

Cortical and trabecular bone Pb measurements were obtained in evaluations performed between 1991 and 1995 in 709 participants in the Normative Aging Study (Wu et al., 2003a). Lead dose was assessed with blood, tibia, and patella Pb concentrations. Renal outcome measures included serum creatinine and estimated creatinine clearance. Mean blood, tibia and patella Pb levels were 6.2 μg/dL (SD 4.1), 22.0 μg/g bone mineral (SD 13.4), and 32.1 μg/g bone mineral (SD 19.5), respectively. After adjustment, analyses in the 670 participants from whom these data were available, revealed a significant inverse association between patella Pb and creatinine clearance ($\beta = -0.069$ [SE not provided]). A borderline significant ($p = 0.08$) inverse

association between tibia Pb and creatinine clearance was also observed. None of the Pb measures were significantly associated with serum creatinine.

Tsaih et al. (2004) reported associations between baseline Pb dose and change in serum creatinine in 448 men. Lead dose was assessed in terms of blood, tibia, and patella Pb. Serum creatinine was measured at baseline and at follow-up, an average of 6 years later. Six percent and 26% of subjects had diabetes and hypertension, at baseline, respectively. Mean blood Pb levels and serum creatinine decreased significantly over the follow-up period in the group. Lead dose was not associated with change in creatinine in all participants. However, a significant interaction was found between blood and tibia Pb and diabetes on change in serum creatinine. For ln blood Pb, $\beta = 0.076$ (95% CI: 0.031, 0.121) in diabetics compared to $\beta = 0.006$ (95% CI: −0.004, 0.016) in non-diabetics. A similar relationship was observed for tibia Pb. An interaction was also observed between tibia Pb and hypertension, although it is possible that many of the 26 diabetics were also included in the hypertensive group and were influential there as well.

6.4.4.1.3 NHANES III

Muntner et al. (2003) analyzed associations between blood Pb and renal outcomes in 15,211 adult subjects enrolled in the NHANES III study, conducted from 1988 through 1994. Dichotomous renal outcome measures analyzed included elevated serum creatinine and chronic kidney disease (GFR < 60mL/min/1.73 m^2). Due to an interaction between blood Pb and hypertension, the population was stratified. Mean blood Pb level was 4.21 µg/dL in the 4,813 hypertensives and 3.30 µg/dL in normotensives. The prevalence of elevated serum creatinine in hypertensives and nonhypertensives was 11.5% and 1.8%, respectively, but the prevalence of chronic kidney disease was similar. The odds ratios for both renal outcomes increased by quartile of blood Pb among the hypertensive subjects but not among those without hypertension. Among those with hypertension, after adjustment for age, race and gender, the odds ratios for elevated creatinine in quartiles 2, 3, and 4 compared to the lowest quartile of blood Pb, were 1.56 (95% CI: 1.04, 2.35), 1.68 (95% CI: 1.24, 2.26), and 2.07 (95% CI: 1.26, 3.40), respectively. The odds ratios were the same following additional adjustment. The authors noted that the "associations were strong, dose-dependent and consistent before and after comprehensive adjustment." They also noted that in nonhypertensives, higher blood Pb was associated with a higher prevalence of chronic kidney disease in diabetics. This study is notable

for sample size, comprehensive adjustment for other renal risk factors, and the fact that this study population is representative of the U.S. non-institutionalized, civilian population.

6.4.4.1.4 Women's Health in the Lund Area Study

In a study of 820 women (age 53 to 64 years) in Sweden, significant negative associations were observed between blood Pb and both GFR (estimated from serum cystatin C) and creatinine clearance (estimated by the Cockcroft-Gault equation [Cockcroft and Gault, 1976]) (Akesson et al., 2005). Mean blood Pb was only 2.2 µg/dL; the association was apparent over the entire dose range (Akesson, 2006). This study has the additional advantage of blood and urinary Cd assessment.

6.4.4.1.5 Summary of Lead-Related Nephrotoxicity in the General Population

General population studies constitute one of the two most important types of research on the renal effects of Pb during the past two decades. Overall, a number of strengths are present in this body of literature. These include study design with longitudinal data in some studies; large populations in both Europe and the United States; comprehensive assessment of Pb dose, including the use of bone Pb as a measure of cumulative Pb body burden in some studies; and statistical approaches that utilize a range of exposure and outcome measures, while adjusting for numerous renal risk factors. Associations between Pb dose and worse renal function were observed in most of the general population studies.

Threshold for Lead-Related Nephrotoxicity

Increased risk for nephrotoxicity has been observed at the lowest Pb dose levels studied to date. Specifically, blood Pb ranged from 2.5 to 3.8 µg/dL in the first significant category in Muntner et al. (2003), and associations between blood Pb as a continuous variable and worse renal function have been reported at a mean of 2.2 µg/dL (Akesson et al., 2005). An association between cumulative Pb dose (mean tibia Pb of 21.5 µg/g bone mineral) and longitudinal decline in renal function has been observed as well, although data on any threshold for this effect were not reported (Tsaih et al., 2004). The data available to date are not sufficient to determine whether nephrotoxicity is related more to current blood Pb levels, higher levels from past Pb

exposures, or both. However, Kim et al. (1996) noted associations in participants whose peak blood Pb levels were ≤10 μg/dL as far back as 1979.

Alternative Explanations for Observed Associations

Potential residual confounding as a possible explanation for associations between Pb dose and adverse health effect outcomes is always a consideration. One general population study provided data useful to address this concern in the Pb-renal literature. For both renal outcomes assessed, Muntner et al. (2003) observed that the odds ratios in hypertensives initially adjusted for age, race, and gender, increased further after additional adjustment for diabetes, systolic blood pressure, smoking status, history of cardiovascular disease, body mass index, alcohol consumption, household income, education level, marital status, and health insurance.
In contrast, after adjustment, regression coefficients decreased in Wu et al. (2003b). However, the analyses were performed in slightly different populations, making interpretation of the adjustment differences less certain. Further, as noted in the Agency for Toxic Substances and Disease Registry's Draft Toxicological Profile For Lead ([2005] Atlanta, GA: U.S. Department of Health and Human Services), since increased blood pressure is associated with Pb dose in general populations, adjustment for hypertension or blood pressure, although extremely common in Pb-renal studies, risks underestimating the actual slope of the association between Pb dose and renal dysfunction. Overall, one of the strengths of the Pb-renal general population literature is the number of factors adjusted for. Thus, residual confounding is an unlikely explanation for observed associations.

Reverse causality has also been considered as a possible explanation for associations between lower blood Pb levels (e.g., <10 μg/dL) and worse renal function (Staessen et al., 1992). Reverse causality attributes increased Pb dose to reduced Pb excretion as a consequence of renal insufficiency. The temporal relation between Pb dose and renal function decline is a critical factor in determining causality. This can be assessed in longitudinal observations of participants with mean blood Pb levels in this lower dose range. Two analyses of longitudinal data from the Normative Aging Study population have been published to date (Kim et al., 1996; Tsaih et al., 2004). Lead dose predicted subsequent decline in renal function over follow-up periods ranging from 3 to 6 years. This was observed even after adjustment for renal function at the beginning of the follow-up period. Longitudinal studies in patients with renal insufficiency have reported

similar findings. Both blood and EDTA-chelatable Pb levels at baseline were significantly associated with decline in estimated GFR over a 4 year follow-up period in 121 patients, even after adjustment for a wide range of covariates, including baseline renal function (Yu et al., 2004) (discussed in Section 6.4.4.3). The same was true in a larger study of 202 chronic renal insufficiency patients over a 2-year follow-up period (Lin et al., 2003). Notably, in both studies, EDTA-chelatable Pb levels were <600 μg/72 h in all participants, with means well below this traditional cut-point. The PheeCad study (the 1990-95 follow-up to the Cadmibel study) appears to have collected relevant data, but the Pb data were not reported in the publication (Hotz et al., 1999).

Biologically, reverse causality should be most prominent in populations with renal insufficiency for a prolonged period of time. However, Kim et al. (1996) observed that blood Pb was positively associated over the entire serum creatinine range, most of which was normal in this general population study and where a substantial decrease in Pb excretion was unlikely. Further, in reverse causality, urinary excretion of Pb should decrease as renal function declines. Urine Pb is not a commonly used Pb dose biomarker, so data from the lower Pb exposure studies are generally not available to assess this. However, higher urine Pb was associated with lower estimated creatinine clearance in Swedish women (Akesson, 2006). Finally, the positive impact of Pb chelation on renal function (discussed in Section 6.4.4.3) may provide evidence against reverse causality. However, the possibility of a direct beneficial effect of the chelating agent on renal function cannot be excluded as an explanatory factor (Gonick et al., 1996). In summary, several lines of evidence suggest that reverse causality is not likely to be a major explanatory factor accounting for observed associations between Pb dose and renal dysfunction.

Consistency of the Magnitude of Associations

Slopes of the associations between blood Pb and creatinine clearance in the general population studies that provide data relevant for such a comparison are shown in Figure 6-8. Since these studies generally had mean blood Pb levels less than 10 μg/dL, slopes of the reported relations were estimated at a blood Pb level of 5 μg/dL. Measured or estimated creatinine clearance data were used from those studies that reported relations for those outcomes. For studies that only reported data for serum creatinine, the slope at a blood Pb of 5 μg/dL was estimated and then the slope was converted to a creatinine clearance slope using the

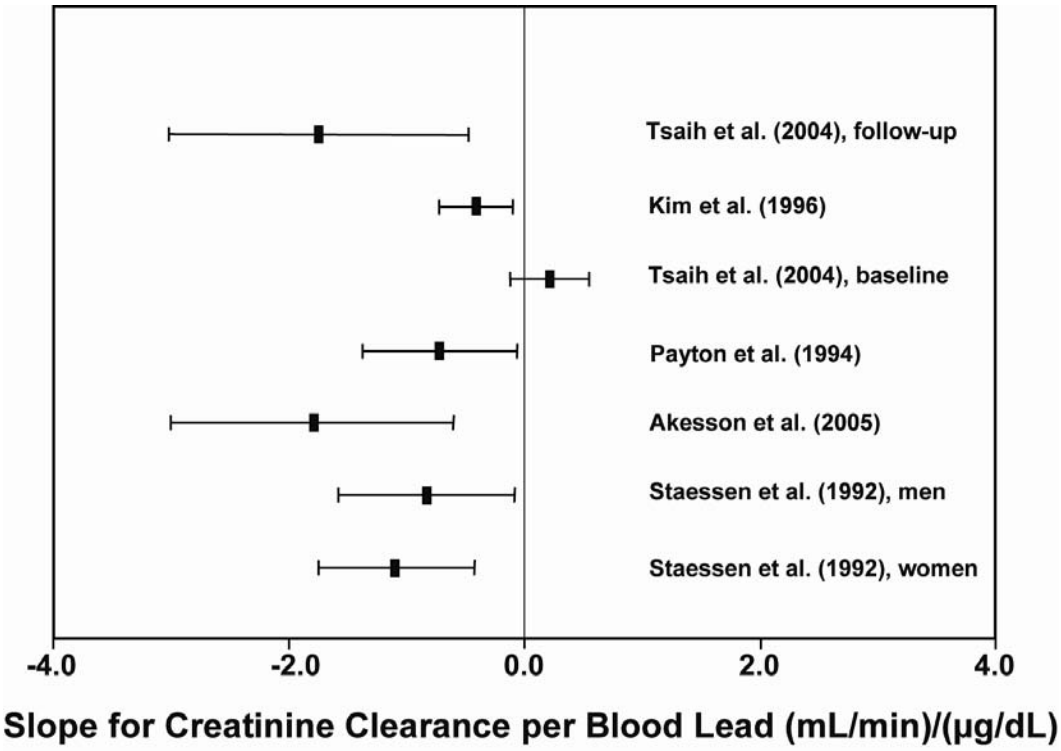

Figure 6-8. Creatinine clearance versus blood lead slope at a blood lead of 5 µg/dL.

Cockcroft-Gault equation (Cockcroft and Gault, 1976). Publication bias may impact the data available for this figure. No significant associations between blood Pb and renal function were observed in two of the general population studies; beta coefficients were not reported (Wu et al., 2003a; de Burbure et al., 2003). However, since Wu et al. (2003a) observed a significant association between patella Pb and creatinine clearance, the study is consistent with results in the majority of the other general population studies. Lastly, a third study (Pocock et al., 1984) reported only that the correlation coefficient between crude blood Pb and serum creatinine was 0.0. Furthermore, publications derived from evaluation of the Normative Aging Study population outnumber those from other populations. Slopes ranged from 0.2 to −1.8 mL/min change in creatinine clearance per µg/dL increase in blood Pb.

Clinical Relevance

It is now clear that chronic kidney disease (CKD) at earlier stages than those requiring actual renal dialysis or transplantation represents a risk factor for cardiac disease and other

causes of mortality and morbidity (Levey et al., 2003). The clinical relevance of the Pb effect can be estimated from the study by Akesson et al. (2005), in which the 5th and 95th percentile values for blood Pb were reported. An increase in blood Pb from the 5th to the 95th percentile (3.5 µg/dL) has the same adverse impact on glomerular filtration as an increase of 4.7 years in age or 7 kg/m^2 in body mass index, both of which are known renal risk factors. In populations at high risk for Pb exposure, a 10-fold increase in blood Pb (e.g., from 1 to 10 µg/dL) would result in an 16.2 mL/min decrease in estimated creatinine clearance or a 22.5% decrease from the mean (Akesson et al., 2005). Sixteen and 9% declines due to a 10-fold increase in blood Pb were predicted based on data for women (Staessen et al., 1992) and men (Payton et al., 1994), respectively. Although Pb exposure is higher in rapidly industrializing countries, high risk populations remain in the United States. In populations with lower blood Pb levels, a downward shift in renal function of the entire population due to Pb may not result in CKD in identifiable individuals; however, that segment of the population with the lowest renal reserve may be at increased risk for CKD when Pb is combined with another renal risk factor. The potential public health importance of population shifts is discussed by the American Thoracic Society (2000) and Rose and Day (1990). Data in both general and patient populations support this concept for Pb exposure. Of note, the above estimates are for general populations. Effect estimates for susceptible populations, such as those with diabetes, hypertension, or chronic renal insufficiency from non-Pb related causes, are likely to be higher.

At-risk Populations

Susceptible populations include those with other risk factors for renal disease, including hypertension, diabetes, and renal disease from other causes. Lead-exposed populations also at increased risk for obesity, diabetes, and hypertension represent groups likely to be the most impacted by Pb exposure. Frequently, both Pb and other risk factors are present in the same lower SES status groups.

In conclusion, the general population literature on the adverse renal effects of Pb benefits from a number of strengths. The consistent associations observed in the majority of these studies provide strong evidence indicating that Pb is a contributor to renal dysfunction in susceptible populations at much lower Pb exposure levels than those previously identified based on data available at the time of the 1986 Lead AQCD.

6.4.4.2 Occupational Studies

The vast majority of studies in the Pb-renal literature were conducted in the occupational setting. This was especially true prior to the 1986 Lead AQCD, but is still also currently the case. Occupational studies of the renal effects of Pb are presented in Annex Table AX6-4.2. In contrast to the general population research discussed above, research on the adverse renal effects of occupational Pb exposure is much less consistent. This is puzzling, since most dose-response relations are thought to be linear. Therefore, biologically, notably elevated Pb doses (as indexed by 30-50 μg/dL blood Pb levels) should be nephrotoxic if lower doses are. Several explanations for this seeming inconsistency are possible. Some are unique to the occupational literature, such as smaller sample sizes. In addition, employed workers are typically healthier and younger than the general population—resulting in the healthy worker bias. This is a particular problem as susceptible risk groups are identified. Survivor bias in cross-sectional studies is also a concern, since workers whose renal function has declined are generally removed from exposure, particularly if they are followed in a medical surveillance program. Few studies have included former workers. Also, statistical analyses have been more limited in occupational studies. Analyses for some outcomes were limited to comparisons between exposed workers and controls whose Pb levels were in the range associated with adverse renal outcomes in environmental work. Use of multiple linear regression has generally involved more limited adjustment for covariates than in most of the environmental studies. Many of these limitations result in bias towards the null, which increases the risk that true associations may not be detected.

Other limitations are pertinent for research on the adverse renal effects of Pb exposure in any population. These factors are likely to have a greater impact on the validity of studies in which one or more of the biases discussed above are also present. These include the insensitivity of the clinical renal outcomes and the lack of uniformly accepted early markers of renal damage in Pb exposure. Limited Pb exposure assessment may also be a factor. Finally, Pb appears to be able to induce an element of hyperfiltration in some settings. Hyperfiltration is a process initially observed in diabetes but is also implicated in other settings, including hypertension and obesity (Nenov et al., 2000). In this process, initial supranormal renal function is paradoxically associated with increased risk for subsequent renal dysfunction. Several occupational studies have reported statistically significant higher mean creatinine clearance in Pb-exposed workers

compared to controls and/or positive associations between higher Pb dose and lower BUN, serum creatinine and/or higher creatinine clearance (Roels et al., 1994; Weaver et al., 2003a, 2005a; Hsiao et al., 2001). Hu (1991) has also reported increased mean creatinine clearance in 22 adults who were Pb poisoned as children, as compared to matched controls (discussed in Section 6.4.5.1), and a recent study reported higher blood Pb to be associated with lower serum creatinine and cystatin C in a study of 800 European children (discussed in Section 6.4.5.3). Longitudinal data for Pb-exposed rodents (discussed in Section 5.7.4.2) are critical in relating this process to Pb. However, in that work, despite similar initial hyperfiltration, subsequent renal dysfunction was much more severe in the high-dose Pb-exposed rodents compared to the low-dose animals. This suggests that hyperfiltration may be one, but not the only, mechanism underlying adverse renal effects of Pb. Whether hyperfiltration contributes to pathology in humans is unclear; longitudinal studies are needed. Regardless, the issue for risk assessment is that significant findings could be obscured if opposite direction associations are present in different segments of the study population and interaction models are not performed to address this.

In the work of Weaver et al. (2003a), no associations were observed when the entire population was studied by several models; however, when interaction models using age as the effect modifier were evaluated, significant associations in opposite directions were observed. This is illustrated in Figure 6-9. This is a valid concern for risk assessment, since the factors involved in these inverse associations in Pb-exposed populations are not well defined at present. Weaver and colleagues have used age as the effect modifier; however, other factors, such as Pb job duration, may be important as well.

In conclusion, a number of limiting factors are observed in the body of research on occupational Pb exposure and adverse renal outcomes. Most of these factors increase the risk that true associations will be missed (bias towards the null). Moreover, Pb appears to have a paradoxical effect on the kidney that further increases this possibility. As a result, the more consistent body of literature in general populations at current Pb exposure conditions provide an appropriate data base for assessing potential renal effects.

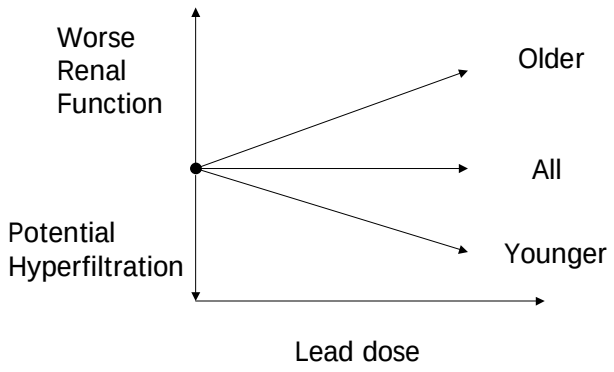

Figure 6-9. Effect on associations between lead dose and renal function depending on whether effect modification (age in this example) is assessed.

6.4.4.3 Patient Population Studies

Studies in various patient populations have also contributed to the body of knowledge concerning adverse renal impacts of Pb exposure (summarized in Annex Table AX6-4.3). Populations studied include those with chronic renal insufficiency (CRI), end-stage renal disease (ESRD), gout, and hypertension, since these diseases are thought to be increased by high-level Pb exposure, particularly when two or more coexist in the same patient. Early research focused on patients with potential Pb nephropathy; and Pb body burdens of interest, assessed with EDTA chelation, were above 600 to 650 µg/72 h. These studies suggested that chelation might be beneficial in Pb nephropathy (Morgan, 1975; Wedeen et al., 1979).

Recurring concerns in this work are, first, whether Pb body burden is higher in all patients with renal insufficiency or failure due to decreased Pb excretion (reverse causality); and, second, whether EDTA-chelatable Pb levels, when measured over a 72-h period in patients with CRI, can be equated to those in participants with normal renal function measured over 24 h. It is possible that, due to decreased excretion of EDTA in renal insufficiency, more Pb per dose is ultimately chelated.

Chelation also may have a direct beneficial effect on kidney function, regardless of Pb exposure, since DMSA has been reported to prevent renal damage in a non-Pb-exposed rat model of nephrosclerosis (Gonick et al., 1996). If so, the benefits of chelation do not appear to

occur via reversal of structural damage (Khalil-Manesh et al., 1992); improved hemodynamics from reduction of reactive oxidant species may be a mechanism (Gonick et al., 1996).

In one of the key studies, Yu et al. (2004) followed 121 patients over a 4-year period. Eligibility required well-controlled CRI. Importantly, serum creatinine between 1.5 and 3.9 mg/dL and EDTA-chelatable Pb <600 µg/72 h were required at baseline. Patients with potentially unstable renal disease were excluded (i.e., due to systemic diseases such as diabetes). Mean age of the study population was 57 years. Mean blood Pb and EDTA-chelatable Pb levels were 4.2 µg/dL and 99.1 µg/72 h, respectively. In a Cox multivariate regression analysis, chelatable Pb was significantly associated with overall risk for the primary endpoint (doubling of serum creatinine over the 4-year study period or need for hemodialysis). The hazard ratio for each 1 µg chelatable Pb was 1.01 (95% CI: 1.00, 1.01; $p = 0.002$). Of the many traditional renal risk factors adjusted for in these models, only the diagnosis of chronic interstitial nephritis was significantly associated with an increase in GFR. Associations between baseline chelatable Pb or blood Pb level and change in GFR (estimated by an MDRD equation [Levey et al., 1999]) were modeled separately using GEE. Based on these models, a 10 µg higher chelatable Pb level or 1 µg/dL higher blood Pb level reduced the GFR by 1.3 and 4.0 mL/min, respectively, during the 4-year study period. This work supports results observed for general populations by suggesting that Pb is nephrotoxic in susceptible populations at lower levels than currently appreciated.

6.4.4.4 Mortality Studies

As summarized in Steenland et al. (1992), mortality studies have consistently shown excess mortality from chronic kidney disease in Pb workers. This increased risk has been most apparent in workers exposed in earlier time periods, becoming nonsignificant in later calendar time periods in a number of studies. Steenland et al. (1992) reported similar results in a study of 1990 former Pb smelter workers. This cohort was made up of predominantly White men who had worked in a Pb-exposed department for at least 1 year between 1940 and 1965. Mean (SD) blood Pb, measured in 1976 in 173 members of this cohort, was 56.3 µg/dL (12.9). There were 8 deaths from chronic kidney disease. Compared to the U.S. White male population, the standardized mortality ratio was 1.26 (95% CI: 0.54, 2.49). The standardized mortality ratio increased with duration of exposure from 0.79 in Pb workers exposed 1 to 5 years to 2.79 in workers exposed for >20 years, although the standardized mortality ratios did not reach

statustical significance (CI not reported). Lead exposure in U.S. industries has declined over the years, and this has been hypothesized as an explanation for the reduction in mortality from renal disease observed in this type of study. However, that fact that improved treatments for chronic renal disease have led to a decrease in mortality from end-stage renal disease (U.S. Renal Data System, 2004) may also be an important factor. The mortality studies by Steenland et al. (1992) and others are described further in Annex Table AX6-4.4.

6.4.5 Lead Nephrotoxicity in Children

6.4.5.1 Studies in Adults Following Childhood Lead Poisoning

Henderson clearly established an increased risk for Pb nephropathy in adult survivors of untreated childhood Pb poisoning (Henderson, 1955). Lead nephropathy was responsible for substantial mortality in the Queensland, Australia population. However, as noted in the 1986 Lead AQCD, other studies of adults who survived childhood Pb poisoning have not reported this degree of renal pathology. Studies published since 1986 are presented in Annex Table AX6-4.5 and also have not observed the degree of renal pathology noted in the Queensland work. Chelation when Pb poisoning was diagnosed may be an explanatory factor in some of these studies.

A study comparing 21 adults, who had experienced childhood Pb poisoning between 1930 and 1942, to age-, sex-, race-, and neighborhood-matched controls found no significant differences in blood Pb level, serum creatinine, or BUN (Hu, 1991). Mean measured creatinine clearance was unexpectedly higher in the previously Pb-poisoned group compared to controls (112.8 versus 88.8 mL/min/1.73 m^2 [$p < 0.01$]). The mean in the Pb-exposed group was also higher than the predicted value of 94.2 mL/min/1.73 m^2 from the nomogram of Rowe et al. (1976). One survivor, who was identified but not included in the study, had been diagnosed with chronic interstitial nephritis on renal biopsy. Her blood Pb was 30 µg/dL, and her presentation was thus consistent with actual Pb nephropathy. Strengths of this study included clear criteria for Pb poisoning and assessment of clinical renal function that included both measured and estimated creatinine clearances. However, the study was limited by small size and the fact that the number enrolled was a very small subset of the initially identified cohort of 192. At least 43 (22.4%) of the 192 were confirmed to be deceased. That group had evidence of higher initial Pb exposure, which raises concern regarding survivor bias in the study group. More importantly,

the higher mean creatinine clearance in the Pb-exposed group provides further evidence for Pb-related hyperfiltration. Again, as discussed in the occupational study section, this may hamper attempts to detect associations between Pb dose and adverse renal effects.

6.4.5.2 Lead Body Burden in Children with Chronic Renal Disease

Schärer et al. (1991) reported higher Pb content in deciduous teeth in 22 German children, age 5 to 14 years, with varying degrees of renal insufficiency compared to a control group of 20 siblings or neighbors and a group of 16 children without known Pb exposure. Mean dental Pb content was 2.8, 1.7, and 1.4 µg/g, in the three groups, respectively. Lead levels in teeth were significantly higher in both the patient and sibling/neighbor control groups compared to the unexposed control group. Mean blood Pb in the renal patients was only 2.9 µg/dL (range 1.1-10.1 µg/dL). Lead in teeth was not correlated with duration of renal impairment.
The authors attributed elevated Pb levels to both exposure and accumulation from decreased renal excretion.

6.4.5.3 Environmental Studies in Children

The insensitivity of the clinical renal outcome measures for early renal damage is a particular problem in children who do not have many of the other renal risk factors, such as hypertension and diabetes, that older adults do. As a result, recent studies in children have favored early biological effect (EBE) markers over clinical renal measures. However, data to determine the predictive value of such biomarkers for subsequent renal function decline in Pb exposed populations are extremely limited. Coratelli et al. (1988) reported a decline in urinary NAG in association with a 1 month period of decreased occupational exposure in 20 adult Pb battery factory workers followed over a 1 year period. Clinical renal function measures were not studied however. Sarasua et al. (2003) studied 526 adults and children, a mean of 4.5 years after an initial evaluation of renal function including measurement of urinary albumin, NAG, RBP, and alanine aminopeptidase. These participants were drawn from three populations exposed to volatile organic compounds and explosives via groundwater and controls. Follow-up was performed to determine if the EBE markers remained elevated and whether the presence of elevated EBE markers at baseline was associated with abnormalities in serum creatinine, serum cystatin C, 24 h creatinine clearance, and urine osmolality at follow-up. Among children who

had elevated EBE markers at baseline, renal EBE markers remained elevated in 38%. However, none remained elevated in the 32 who had completed adolescence by the time of the follow-up. The authors noted the potential for puberty related biomarker changes. Also, abnormalities in the clinical measures were rare at follow-up.

The environmental studies in children generally focused on children living near industrial sources and controls. These studies are summarized in Annex Table AX6-4.5. Three studies that included analysis of clinical renal outcomes are of note. Fels et al. (1998) found no difference in mean serum creatinine between 62 exposed and 50 control children; correlations, if assessed were not reported. Staessen et al. (2001) studied 200 17-year-old Belgian children. The two exposed groups were recruited from industrialized suburbs, whereas the control group was recruited from a rural area. Mean blood Pb levels were 1.5, 1.8, and 2.7 μg/dL in controls, and exposed groups one and two, respectively. Although blood Pb levels were low, after adjustment for sex and smoking status, blood Pb was positively associated with both serum cystatin-C and urinary β_2-microglobulin. Blood Cd was not associated with either outcome. In contrast, De Burbure et al. (2006) observed associations between higher blood Pb and lower serum creatinine and cystatin C in models with 300-600 European children (depending on outcome). The authors considered this to be suggestive of hyperfiltration. Additional research in children, including longitudinal follow-up, is needed.

6.4.6 Mechanisms for Lead Nephrotoxicity

Individuals who have been heavily exposed to Pb are at increased risk for both gout and renal disease (Shadick et al. 2000; Batuman 1993). Lead is thought to increase serum uric acid (urate) by decreasing its renal excretion (Emmerson, 1965; Ball and Sorensen, 1969; Emmerson and Ravenscroft, 1975). As discussed above, research during the past decade indicates that Pb is nephrotoxic at lower levels than previously recognized. The same is true for uric acid (Johnson et al., 2003). Therefore, it is possible that one mechanism for Pb-related nephrotoxicity, even at current lower levels of Pb exposure, is via increasing serum uric acid.

In order to address this question, Weaver et al. (2005a) analyzed data from 803 current and former Pb workers to determine whether Pb dose was associated with uric acid and whether previously reported associations between Pb dose and renal outcomes (Weaver et al., 2003a) were altered after adjustment for uric acid. Outcomes included uric acid, blood urea nitrogen,

serum creatinine, measured and calculated creatinine clearances, and urinary NAG and RBP. Mean uric acid, tibia Pb, and blood Pb levels were 4.8 mg/dL (SD 1.2), 37.2 μg/g bone mineral (SD 40.4), and 32.0 μg/dL (SD 15.0), respectively. None of the Pb measures (tibia, blood, and DMSA-chelatable Pb) were associated with uric acid, after adjustment for age, gender, body mass index, and alcohol use. However, when effect modification by age on these relations was examined, both blood and tibia Pb were significantly associated in participants in the oldest age tertile ($\beta = 0.0111$ [95% CI: 0.003, 0.019] and $\beta = 0.0036$ [95% CI: 0.0001, 0.007]) for blood and tibia Pb, respectively). These models were further adjusted for blood pressure and renal function. Hypertension and renal dysfunction are known to increase uric acid. However, they are also risks associated with Pb exposure. Therefore, adjustment for these variables in models of associations between Pb dose and uric acid likely results in overcontrol. On the other hand, since non-Pb-related factors contribute to both renal dysfunction and elevated blood pressure, lack of adjustment likely results in residual confounding. Therefore, as expected, associations between Pb dose and uric acid decreased after adjustment for systolic blood pressure and serum creatinine, although blood Pb remained borderline significantly associated ($\beta = 0.0071$ [95% CI: −0.001, 0.015]). However, when the population was restricted to the oldest tertile of workers with serum creatinine greater than the median (0.86 mg/dL), likely the highest risk segment of the population, blood Pb remained significantly associated with uric acid even after adjustment for systolic blood pressure and serum creatinine ($\beta = 0.0156$). Next, in models of renal function in all workers, uric acid was significantly associated with all renal outcomes except NAG. Finally, in the oldest tertile of workers, after adjustment for uric acid, associations between Pb dose and NAG were unchanged, but fewer of the previously significant ($p \leq 0.05$) associations noted between Pb dose and the clinical renal outcomes in Weaver et al. (2003a) remained significant.

Data from the Normative Aging Study indicate that Pb dose, at levels lower than those known to increase the risk for gout or in the study of Weaver et al. (2005a), is associated with increased uric acid (Shadick et al., 2000). In 777 participants, mean blood, patella, and tibia Pb levels were 5.9 μg/dL, 30.2 μg/g bone mineral, and 20.8 μg/g bone mineral, respectively. A significant association between patella Pb and uric acid ($\beta = 0.007$ [95% CI: 0.001, 0.013]; $p = 0.02$) was found, after adjustment for age, BMI, diastolic blood pressure, alcohol ingestion, and serum creatinine. Borderline significant associations between tibia ($p = 0.06$) and blood

Pb (p = 0.1) and uric acid were also observed. Notably these associations were significant even after adjustment for blood pressure and renal function, providing further evidence that low-level Pb exposure increases uric acid.

These data suggest that older workers comprise a susceptible population for increased uric acid due to occupational Pb exposure. Uric acid may be one mechanism for Pb-related nephrotoxicity. However, this is not the only mechanism, since in Weaver et al. (2005a), the association between blood Pb and serum creatinine remained significant even after adjustment for uric acid. These mechanistic relations have more than just theoretical importance. Clinically relevant therapies may be possible since EDTA chelation has been reported to improve both renal function and urate clearance in patients with renal insufficiency and gout, even when EDTA-chelatable Pb body burdens were low (Lin et al., 2001b).

6.4.7 Susceptible Populations for Lead Nephrotoxicity

6.4.7.1 Chronic Medical Diseases

The general population studies by Tsaih et al. (2004) and Muntner et al. (2003) (discussed in Section 6.4.4.1) indicate that patient populations with diabetes and hypertension are at increased risk for adverse renal effects of Pb. Lin et al. (2001a, 2002) indicate that patients with CRI and gout are also at increased risk. In these settings, Pb appears to acts as a cofactor with other renal risk factors to cause early onset of renal insufficiency and/or a steeper rate of renal function decline. It is likely that the presence of larger high risk populations within general populations is an important factor in the lower Pb dose thresholds noted for the adverse effects of Pb on the kidney in environmental compared to occupational research.

6.4.7.2 Age

Weaver et al. (2003a, 2005a,b) found older age to be a risk factor for adverse renal effects in Korean Pb workers. This is consistent with research in general populations (Lindeman et al., 1985) and is biologically plausible, since most renal risk factors increase with age. Gonick and Behari (2002) have summarized the data regarding the potential contribution of Pb exposure to essential hypertension; similar issues may be involved with the renal dysfunction observed in aging.

6.4.7.3 Genetic Polymorphisms

Research in the last two decades suggests that several genetic polymorphisms affect Pb toxicokinetics (i.e., modify the relation between Pb exposure and dose). Of those potentially relevant to the kidney, data on the gene that encodes for ALAD are the most important in this regard. The ALAD enzyme is a principal Pb-binding protein; the isozymes in those with the ALAD2 allele are more electronegative and bind a greater proportion of blood Pb than does the protein in individuals with the ALAD 1-1 genotype (Bergdahl et al., 1997). Research to date indicates that individuals with the ALAD2 allele generally have higher blood Pb levels than those with the ALAD 1-1 genotype, although this may not be the case at lower levels of Pb exposure (i.e., mean blood Pb levels <10 μg/dL) (Kelada et al., 2001). Participants with the ALAD2 allele have been found to have lower bone Pb levels in some studies (Hu et al., 2001; Kamel et al., 2003); other toxicokinetic differences have also been reported (Fleming et al., 1998; Hu et al., 2001; Schwartz et al., 1997; Smith et al., 1995). Overall, these data suggest that tighter binding of Pb by the isozymes of the ALAD2 allele decreases Pb sequestration in bone.

In contrast, data to determine whether the ALAD polymorphism impacts the renal toxicity of Pb are still quite limited. The only environmentally exposed population in which this has been addressed is the Normative Aging Study. Wu et al. (2003a) (discussed in detail in Section 6.4.4.1.2) analyzed data to determine whether the ALAD genetic polymorphism modified associations between Pb dose and uric acid, serum creatinine, and estimated creatinine clearance. A total of 114 (16%) of the study group were either homozygous or heterozygous for the variant ALAD2 allele. None of the three outcomes were significantly different by genotype. However, effect modification by genotype on the association between tibia Pb and serum creatinine was observed; the β coefficient (and slope) was greater in the group with the variant allele ($\beta = 0.002$ [SE not provided]; $p = 0.03$). Effect modification of borderline significance ($p < 0.1$) for relationships between patella or tibia Pb and uric acid was observed; this was significant in participants whose patella Pb levels were above 15 μg/g bone mineral ($\beta = 0.016$ [SE not provided]; $p = 0.04$). Similar to the serum creatinine model, patella Pb was associated with higher uric acid in those with the variant allele. Genotype did not modify Pb associations in models of estimated creatinine clearance.

The impact of the ALAD polymorphism on renal outcomes has been studied in four occupationally-exposed populations to date. The two that assessed both associations and effect

modification by genotype are discussed here. Weaver et al. (2003b) analyzed data from 798 Pb workers. A total of 79 (9.9%) participants were heterozygous for the ALAD2 allele (none was homozygous). After adjustment, participants with the ALAD2 allele had lower mean serum creatinine and higher calculated creatinine clearance. Effect modification by ALAD on associations between blood Pb and/or DMSA-chelatable Pb and three of six renal outcomes was observed. Among those with the ALAD 1-2 genotype, higher Pb measures were associated with lower BUN and serum creatinine and higher calculated creatinine clearance. Among older workers (age ≥ median of 40.6 years), ALAD genotype modified associations between Pb dose and uric acid levels. Higher Pb dose was significantly associated with higher uric acid in workers with the ALAD 1-1 genotype; associations were in the opposite direction in participants with the variant ALAD 1-2 genotype (Weaver et al., 2005c).

Ye and colleagues (2003) assessed effect modification by ALAD on associations between blood Pb with urinary NAG and albumin in a study of 216 Pb workers. Geometric mean blood Pb was 37.8 µg/dL in 14 workers with the ALAD 1-2 genotype and 32.4 µg/dL in workers with the ALAD 1-1 genotype. After adjustment for age, NAG was borderline statistically higher in those with the variant allele whose blood Pb levels were ≥40 µg/dL. In all Pb workers, after adjustment for age, gender, smoking, and alcohol ingestion, a statistically significant positive association between blood Pb and creatinine adjusted NAG was observed in the workers with the ALAD 1-2 genotype but not in Pb workers with the ALAD 1-1 genotype (the groups were analyzed separately rather than in an interaction model).

Thus, two of the three studies reported steeper slopes for one or more associations between Pb dose and adverse renal function in participants with the ALAD2 allele compared to those with the ALAD 1-1 genotype, which suggests that the variant ALAD gene confers additional risk for adverse renal outcomes in Pb-exposed populations. If the associations of Weaver et al., (2003b) represent Pb-induced hyperfiltration, their results could be consistent with increased risk from the variant allele as well. Ultimately, analysis of longitudinal data in the Korean Pb worker population will be needed to understand these complex relationships.

6.4.8 Confounding of the Renal Effects of Lead by Other Potential Risk Factors

Studies selected for discussion in Section 6.4 above have generally controlled for at least the most basic risk factors known to affect renal function, such as age, gender, and body mass index (or weight and height separately). Some have controlled for many other potentially important risk factors. In addition, exposure to other nephrotoxicants must be considered. Notably, although these are listed under confounders, some may be effect modifiers as well.

6.4.8.1 Cadmium

Similar to Pb, cadmium (Cd) is an ubiquitous nephrotoxicant that accumulates in the body. Environmental exposure to Cd in the United States occurs primarily through food and smoking (Agency for Toxic Substances and Disease Registry, 1993). Cadmium in food is a result of soil pollution from a variety of human activities such as phosphate fertilizer use, industrial releases from smelting, and fuel combustion. An analysis of NHANES III data, collected in a representative sample of the U.S. population from 1988-1994, indicates that mean urinary Cd is 0.48 μg/g creatinine, and 97.7% of the population has a level ≤2.0 μg/g creatinine (Paschal et al., 2000). Also similar to Pb, Cd causes proximal tubule pathology and is a known risk factor for chronic renal insufficiency (CRI).

Existing data indicate that Cd, at exposure levels common in the United States, confounds associations between Pb exposure and at least one renal outcome, NAG. Roels et al. (1994) reported higher mean NAG in their Pb-exposed group; however, NAG was correlated with urinary Cd but not blood or tibia Pb, despite mean urinary Cd being only 1.04 and 0.53 μg/g creatinine in workers and controls, respectively. Cardenas et al. (1993) reported a similar finding. Bernard et al. (1995a) found an association between urinary Cd and the NAG-B isoenzyme (released with breakdown of proximal tubular cells) in 49 Cd workers and 20 age-matched controls. In multiple linear regression, urinary Cd, but not Pb, was associated with NAG-B after adjustment for age. The association was significant even in the 44 participants with Cd levels <2 μg/g creatinine. However, NAG-A (released by exocytosis) was correlated with urinary Pb (the only Pb measure), but not Cd. Roels et al. (1995) reviewed data pertinent to the potential for Cd confounding of associations between Pb and NAG. In more recent work, Weaver et al. (2003a) measured urinary Cd in a subset of 191 of the 803 workers in their study

(mean urinary Cd was 1.1 μg/g creatinine). Higher urinary Cd levels were associated with higher NAG. Of the Pb measures obtained, only tibia Pb was significantly associated with NAG in the Cd subset. When urinary Cd and tibia Pb were entered as covariates in the same model, both remained associated with NAG ($p < 0.05$). However, in comparing the effects, a 0.5 μg/g creatinine increase in Cd had the same effect on NAG as a 66.9 μg/g bone mineral increase in tibia Pb. When compared by ranges of exposure in this population, environmental level Cd dose had a larger impact on NAG than did occupational Pb dose.

Cadmium exposure may well confound relations between Pb exposure and other renal outcomes as well, but available data are too limited to draw firm conclusions. Positive associations between urinary Cd, which is thought to be the best measure of cumulative Cd exposure in the absence of Cd-related renal damage, and low molecular weight (LMW) proteinuria are well established in the occupational setting. LMW proteinuria, most commonly assessed by β_2-microglobulin, is generally progressive at Cd levels >1500 μg/g creatinine in workers with substantial body burdens (one or more historical urinary Cd >20 μg/g creatinine) but may also be progressive at lower levels (Roels et al., 1997; Bernard, 2004). More importantly, clinical renal function also declines as evidenced by decreasing GFR in Cd-exposed workers followed longitudinally after removal from exposure due to LMW proteinuria (Roels et al., 1989; 1997).

In contrast to the clear evidence that Cd is a renal toxicant at occupational levels of exposure, the renal risk from lower level Cd exposure remains uncertain. Most studies of environmental Cd exposure are cross-sectional and have assessed EBE markers, rather than clinical renal outcomes (Alfvén et al., 2002; Järup et al., 2000; Noonan et al., 2002; Olsson et al., 2002). The Cadmibel study, a general population study of exposed residents from both Cd-polluted and unpolluted areas (discussed in Section 6.4.4.1.1), found correlations between urinary Cd and several urinary EBE markers (NAG, RBP, β_2-microglobulin, calcium, and amino acids) (Buchet et al., 1990). In those models, after adjustment for urinary Cd and other covariates, blood Pb was significant in models of β_2-microglobulin and amino acids but not NAG. However, in this same population, blood Pb was inversely associated with creatinine clearance, whereas urinary and blood Cd were not (Staessen et al., 1992). A 5-year follow-up was conducted to determine the significance of the EBE abnormalities (Hotz et al., 1999). In this study, models of renal function (two dichotomized outcomes: a 20% decline in creatinine

clearance and a 20% increase in albumin excretion) in relation to quartiles of urinary Cd and the EBE markers at baseline were analyzed by likelihood ratios. Baseline variables did not predict adverse renal outcomes. However, 25% of the original population was lost to follow-up; available data indicated that their baseline renal function was worse than those who participated in the follow-up study. This may have biased the study towards the null.

Three recent publications suggest that low-level Cd exposure is associated with adverse clinical renal outcomes. Elevated urine Cd levels were associated with decreased calculated creatinine clearance and with prevalent microalbuminuria after adjustment for age, sex, race, smoking, and use of diuretics in an analysis of 16,094 participants in the NHANES III study (Young et al., 2004). Also, Hellström et al. (2001) reported increased rates of renal dialysis and transplantation in residents of Cd-polluted areas in Sweden. Compared to the "no exposure group" (domicile >10 km from a battery plant), age-standardized rate ratios were 1.4 (95% CI: 0.8, 2.0) in the low-exposure group (domicile 2 to 10 km) and 1.9 (95% CI: 1.3, 2.5) in the moderate-exposure group (domicile <2 km). Exposure categorization was based on environmental monitoring in the study areas. Cadmium dose was not directly measured, although occupationally exposed participants were considered in a separate group. The third study, by Åkesson et al. (2005), also assessed Pb exposure as a covariate, an important approach given the Cadmibel results (Staessen et al., 1992). Blood and urinary Cd were associated with worse GFR and creatinine clearance. The association for blood Cd and decreased creatinine clearance remained statistically significant even in non-smokers, suggesting that a public health remedy, in addition to smoking cessation, may be of value.

In conclusion, Cd clearly confounds associations between Pb dose and NAG. Given the similarities in both nephrotoxicants, Cd may confound and/or modify associations between Pb and other renal outcomes. However, data regarding the concentration-response relationship between environmental Cd and the kidney are too limited to assess the potential for this at present. Future studies assessing both Pb and Cd are needed.

6.4.9 Summary of the Epidemiologic Evidence for the Renal Effects of Lead

During the past two decades, the quality of research on the renal impacts of Pb exposure has advanced dramatically. As a result, a much more accurate assessment of the adverse renal impact of Pb exposure can now be made. General population studies are the most important

advance in this regard. These studies provide strong evidence that renal effects occur at much lower blood Pb levels than previously recognized. These effects are clinically relevant in U.S. subpopulations who continue to have higher Pb exposure than the general population. At levels of exposure in the general U.S. population overall, Pb combined with other risk factors, such as diabetes, hypertension, or chronic renal insufficiency from non-Pb related causes, can result in clinically relevant effects. Notably, the size of such susceptible populations is increasing in the United States due to obesity.

- The majority of studies in general adult and patient populations published during the past two decades have observed associations between Pb dose and worse renal function. Other explanations, such as residual confounding or reverse causality, are less likely. The renal effects of Pb on children are difficult to assess, as most of these studies only measured early biological effect markers which have unknown clinical significance.

- The magnitude of the effect of Pb on renal function ranged from 0.2 to –1.8 mL/min change in creatinine clearance per μg/dL increase in blood Pb in general population studies. The size of the effect was relatively consistent across the studies, although only five provided data useful for this determination (three were at different time points in the Normative Aging Study population) and a form of publication bias may be present in studies that provided no data and only reported that associations were not significant. One patient population (individuals with CRI) study reported a similar effect of blood Pb longitudinally on yearly decline in GFR.

- The cumulative effect of higher blood Pb levels from past exposure may be a factor in nephrotoxicity observed at current blood Pb levels. However, one study found associations between blood Pb and concurrent serum creatinine in participants whose peak blood Pb levels were ≤10 μg/dL.

- The threshold for Pb-related nephrotoxicity cannot be determined based on current data. However, associations with clinically relevant renal outcomes have been observed in populations with mean blood Pb levels as low as 2.2 μg/dL.

- Research in the occupational setting is far less consistent. However, a notable finding from several of these studies is the observation of inverse associations (higher Pb dose with lower BUN, serum creatinine, and/or higher creatinine clearance). This may indicate Pb-related hyperfiltration and may have mechanistic implications.

6.5 CARDIOVASCULAR EFFECTS OF LEAD

6.5.1 Summary of Key Findings of the Cardiovascular Effects of Lead from the 1986 Lead AQCD and Addendum, and 1990 Supplement

The greater part of the evidence reviewed up to 1990 included analyses of the largest datasets available at the time: (1) the National Health and Nutrition Evaluation Survey II (NHANES II), studying the U.S. population between 1976 and 1980; and (2) the British Regional Heart Study (BRHS), studying men aged 40-59 years from 24 British towns. Analyses of the Welsh Heart Programme, a regional Welsh study, and the Caerphilly Collaborative Heart Disease Study, a cohort study of men aged 45-59 years living in one town in Wales, as well as smaller population and occupational exposure studies in the United States, Canada, and Europe, provided further supporting evidence. These studies set enduring design and analysis standards by example for evaluating cardiovascular effects associated with blood Pb levels in samples from diverse populations.

In general, the reviewed studies used multiple linear regression modeling of blood pressure and multiple logistic regression modeling of hypertension, cardiovascular mortality, and other cardiovascular disease, allowing adjustment of the blood Pb effect on outcome by other factors known or suspected to be related to the exposure and outcome under study. The most commonly considered potential confounding factors were age, body mass index (BMI), alcohol use, and cigarette smoking.

These studies were almost exclusively cross-sectional, measuring cardiovascular outcome, blood Pb, and control variables once, although one Canadian occupational study and one Danish birth-year cohort study used a longitudinal design. Some studies presented analyses stratified by sex or age, by both sex and age, or by race. Other analyses only reported results for one particular stratum. Separate analyses of datasets partitioned by stratified variables always reduce sample size available for statistical models and may thereby reduce power to detect real effects.

Evaluated as a whole, the earlier available blood pressure studies supported a small but significant association between increasing blood Pb concentrations and increasing blood pressure in study groups. The effect was more consistent across studies in middle-aged men than in other groups, ranging from a 1.5 to 3.0 mm Hg increase in systolic blood pressure for each doubling of blood Pb from the mean blood Pb level, and from a 1.0 to 2.0 mm Hg increase in diastolic blood pressure for each blood Pb doubling, across a wide range of blood Pb concentrations down to

7 μg/dL. Most studies using multiple regression analyses stratified by sex did not find significant associations between blood pressure and blood Pb in females, though one reanalysis of the NHANES II dataset did report a statistically significant relationship between diastolic blood pressure and blood Pb in women aged 20 to 74 years. In studies reporting the use of different blood Pb-blood pressure concentration-response relationships, log blood-Pb terms had lower probability values than linear blood-Pb terms, suggesting that increases in blood pressure with fixed increases in blood Pb might be greater at lower than higher blood Pb concentrations.

Three studies of groups with occupational exposure reported mixed results. One study found significant excess mortality due to cardiovascular disease during the 1946-1965 period in a case-control study in the United Kingdom, but not during 1966-1985. A study of U.S. battery and Pb production workers from 1947-1980 found significant excess mortality due to "other hypertensive disease" (codes 444-447 in the ICD 1955 classification system), but not due to hypertensive diseases outside those classifications. No excess mortality due to hypertension was found in a study of U.S. smelter workers between 1940 and 1965.

The BRHS study did not find significant associations between blood Pb and ischemic heart disease and stroke, though low power to detect such an effect should be noted. However, electrocardiogram abnormalities associated with left ventricular hypertrophy were found to be related to blood Pb in a subset of the NHANES II data, confirming an earlier study finding significant associations between blood Pb and ischemic changes in Pb workers.

Noninvasive measurement of bone Pb concentration using XRF techniques was still maturing during the literature review period covered by the 1986 Lead AQCD/Addendum and 1990 Supplement. No cardiovascular studies had yet been reported using bone Pb as a marker for Pb exposure. The previous Lead AQCD concluded that there was a small but statistically significant relationship between blood-Pb level and adverse cardiovascular outcome. Future research needs noted were large sample sizes, identification of susceptible populations, more precise quantitative estimation of effect size, and better definition of the dose-response relationship.

6.5.2 Effects of Lead on Blood Pressure and Hypertension

6.5.2.1 Introduction

Blood Pb concentration has remained the most widely used Pb exposure index in blood pressure/hypertension epidemiologic studies from 1990 to present. Obtaining the sample is relatively noninvasive and quick, pertinent measurement techniques are well standardized and inexpensive, there is wide access to external quality assurance programs, and existing regulation and medical decision-making are based on blood Pb levels. If exogenous Pb exposure were the only determinant for blood Pb concentration, it could be fair to state that a single blood Pb measurement reflects exposure to Pb during the 30-90 day period preceding the measurement. However, blood Pb concentration represents a combination of recent exposure to external sources and the influence of internal sources, principally bone Pb. As detailed in Chapter 4, bone is a long-term storage depot for much of the Pb absorbed by the body from external sources and, by weight, can represent over 95% of the total Pb body burden in middle-aged persons, especially where current external exposures are low. Bone Pb has residence times of years to decades. Also, bones constantly absorb Pb from and release Pb back into the circulatory system. Consequently, blood Pb concentration is not only determined by current and recent past external Pb exposure but is also influenced by existing bone Pb concentration to a degree determined by current external exposure, accumulated past exposure Pb stored in bones, and the physiological state of the bones due to aging, disease, pregnancy, and lactation, among other factors. Studies using only blood Pb concentration as an exposure index cannot determine the relative contributions of current exogenous exposure and endogenous exposure to blood Pb. Thus, they are unable to assess what part of measured blood Pb effect on the circulatory system is due to possibly higher long duration past exposure and what part is due to the possibly immediate toxic effects of currently circulating Pb. They are, instead, assessing a combined effect of past and present exposure in a proportion that will differ among subjects according to their past and present exposure, health history, and age.

The newly developed in vivo technique of XRF measurement of bone Pb concentration has been used in a handful of studies to better assess the role of past Pb exposure on blood pressure and hypertension in essentially cross-sectional studies. Bone Pb concentration provides a record of cumulative past exposure due to the long residence times of Pb in bones, though the specific temporal pattern of past exposure cannot be readily determined from the measurement.

Primarily cortical bones (such as tibia) have residence times measured in decades, whereas primarily trabecular bones (such as calcaneus and patella) have Pb residence times measured in years to decades, reflecting different metabolic rates of the two bone types. As there is continual interchange of Pb in bone and Pb in blood, studies combining the measurement and modeling of both bone and blood Pb have the best chance of dissecting out the roles of past and present Pb exposure on blood pressure and hypertension.

Elevated blood pressure can be evaluated as a continuous measure (mm Hg) or as a dichotomized measure (hypertension). The definition of hypertension involves a categorical cut point of mm Hg above which one is hypertensive and below normotensive. Kannel (2000a,b) notes that this number has dropped over time for systolic/diastolic pressure and further notes a continuous graded influence of blood pressure on health even within what is regarded as the normotensive range. The hypertension definition is to some extent arbitrary, as the cut point has changed over time. However defined for any given study, regardless of medical definition for the year of the study, hypertension classification offers a different perspective than blood pressure per se. Hypertension has a different clinical relevance than blood pressure changes themselves. The disease condition as an outcome and a change in mm Hg in relation to exposure both offer the opportunity for insight into the clinical relevance of the relationships. Biomarkers like bone Pb and blood Pb also help to distinguish acute and chronic exposure effects.

Blood pressure is an inherently variable measure. Even when measured with indwelling catheters, blood pressure varies on a minute to minute interval in the same individual. Extrinsic sources of blood pressure variability include measurement technique, the tester, and conditions under which the measurements are taken. All the sources of measurement error are additive, but the expected total error will be symmetrically distributed about some true blood pressure, i.e., unbiased. Under conditions where the size of the expected Pb effect is in the same range as the total measurement error, large studies with high power are required for detecting real effects where they exist. These factors favor studies with large numbers of subjects. Using blood Pb as a surrogate for brain Pb biases the blood Pb regression coefficient towards zero. This is an example of classical measurement error. Smaller blood pressure studies may fail to detect Pb effects simply because of low power. As noted in the 1986 AQCD/Addendum and the 1990 Supplement, stratification of data sets always reduces power.

The growing field of toxicogenetics now includes Pb exposure epidemiology. The several studies combining subject evaluation of polymorphisms of genes thought to play a role in either the origin of cardiovascular disease, the toxicokinetics of Pb, or both are also reviewed here.

6.5.2.2 Blood Pressure and Hypertension Studies Using Blood Lead as Exposure Index

Table 6-2 lists studies showing estimates of the relationship between systolic blood pressure and blood Pb level. The table focuses on the key studies with low blood Pb in the United States to include studies of the general population (NHANES), the Boston NAS, and the international meta-analysis by Nawrot et al. (2002). Studies were included if the population was not all occupationally exposed or limited to women during pregnancy, or children. Effects were included only if they were based on the entire study group or secondly, the subgroup had more than 500 people. Other studies are discussed in the text and presented in Annex Table AX6-5.1.

6.5.2.2.1 NHANES Studies

NHANES contributed the largest datasets analyzed in this review. As the surveys are also representative of the U.S. population, their results may be more readily applied to the general U.S. population than smaller cohort or occupational studies. The several papers using this dataset sometimes come to different conclusions, depending on the statistical techniques used in the analyses, including logarithmic or linear specification of the Pb variable, stratification of analyses according to sex or ethnic groups or use of interaction terms to define these groups, use of survey-design corrected models, choice of covariates in the models, and different age ranges analyzed.

NHANES II (1976-1980)

In one NHANES II-based study, males and females (number unreported but less than 9,000 combined) aged 20 to 74 years were studied with separate stepwise multiple regression models adjusted for sampling design (Schwartz, 1991). Mean blood Pb levels and ranges were not reported. Covariates common to both male and female models were age and age^2, BMI, race, family history, cholesterol, zinc, tricep fold, and natural log Pb. Models for men also included height and cigarette smoking. Natural log blood Pb was significantly associated with diastolic blood pressure (systolic not reported) in males, with a 2.03 mm Hg diastolic (95% CI: 0.67,

Table 6-2. Summary of Studies with Quantitative Relationships of Systolic Blood Pressure and Blood Lead

Reference	Study Location and Gender	n	Blood Lead Arithmetic Mean (25th and 75th Percentiles)	Estimated Slope (95% CI), mm Hg per Change in Blood Lead from 5 to 10 µg/dL
Vupputuri et al. (2003)	NHANES* III White males	5,360	4.4 (1.0, 4.9)	0.43 (-0.36, 1.26)
	White females	5,188	3.0 (0.5, 2.9)	0.52 (-0.74, 1.77)
	Black males	2,104	5.4 (1.2, 6.0)	1.24 (0.29, 2.18)
	Black females	2,300	3.4 (1.0, 4.0)	2.35 (0.71, 4.00)
Den Hond et al. (2002)	NHANES III 1988-94 White males	4,685	3.6 (2.3, 5.3)	0.3 (-0.2, 0.7)
	White females	5,138	2.1 (1.3, 3.4)	0.1 (-0.4, 0.5)
	Black males	1,761	4.2 (2.7, 6.5)	0.9 (0.04, 1.8)
	Black females	2,197	2.3 (1.4, 3.9)	1.2 (0.4, 2.0)
Nash et al. (2003)	NHANES II Females	1,786	2.9 (range 0.5-31.1)	1.60 (0.05, 3.15)
Sorel et al. (1991)	NHANES II Males	2,044	Black: 20.1 White: 16.8	0.40 (-0.15, 0.95)
	NHANES II Females	2,056	Black: 13.2 White: 12.1	0.20 (-1.40, 1.00)
Cheng et al. (2001)	Boston Normative Aging Study Males (about 97% White)	519	5.9 (3.4, 7.4)	-0.16 (-1.67, 1.35)
Proctor et al. (1996)	Boston Normative Aging Study Males	798	6.5 (3.8, 8.1)	0.59 (-0.76, 1.87)
Nawrot et al. (2002)	31 U.S. and European Studies (includes occupationally exposed)	>58,490	Meta-analysis	1.0 (0.5, 1.4)

NHANES—United States population sample.

3.39) increase for every doubling of blood Pb and, for females, a 1.14 mm Hg increase (95% CI: 0.13, 2.08). Interactions between blood Pb and sex and between blood Pb and race in a combined model were insignificant (not shown). The conclusion from these interaction terms is

that the association between blood Pb and diastolic blood pressure was not significantly different between men and women or between races. Stepwise modeling may inflate statistical Type I error.

The other NHANES II-based study focused on Black-White differences in blood pressure related to blood Pb (Sorel et al., 1991). There were 473 Blacks and 3,627 Whites in the study, each divided nearly evenly by sex, aged 18 to 74 years. Blood Pb means and ranges were not given. As is usual in U.S.-based studies, race/ethnicity was based on self-report. Survey design-adjusted multiple regression models were stratified on sex and included age, BMI, and linear blood Pb as covariates. Effects of race and poverty index were assessed by including their terms in models with and without blood Pb and determining change in race or poverty coefficients by comparing confidence intervals. Each 1 μg/dL increase in linear blood Pb significantly predicted increased systolic blood pressure for both males (0.13 mm Hg/μg/dL) and females (0.08 mm Hg/μg/dL), but not diastolic blood pressure. The differences in Black and White (race variable) blood pressure coefficients did not significantly change when Pb was in or out of the model, either for subjects below the poverty index or above the poverty index. Race did not appear to significantly modify the relationship between blood Pb and systolic blood pressure. There were reporting inconsistencies in the female-stratified models, in which the coefficients and 95% CI did not correspond.

NHANES III (1988-1994)

A study using the NHANES III dataset from all adults 20 years of age and up examined the effect of natural log blood Pb on systolic and diastolic blood pressure (Den Hond et al., 2002). Multiple regression analyses for each blood pressure measurement were stratified by sex and race, yielding four models for each blood pressure measurement. The mean Pb blood levels were 3.6 μg/dL for White males (n = 4,685), 2.1 μg/dL for White females (n = 5,138), 4.2 μg/dL for Black males (n = 1,761), and 2.3 μg/dL for Black females (n = 2,197). The overall blood Pb range was < 0.8 to > 20.0 μg/dL. One group of covariates (age, age-squared, BMI, hematocrit, smoking, alcohol consumption, and an indicator variable for use of antihypertensive medications) were first entered as a block regardless of significance in each model. Next, another group of variables (coffee consumption, dietary calcium, dietary sodium/potassium ratio, total serum protein, total serum calcium, diabetes, and poverty index) was entered stepwise into

the model without Pb, and the variable was retained only if it was statistically significant ($p < 0.05$). Then log-transformed blood Pb was forced into each model. The model building procedure resulted in eight distinct models, each with their own unique mix of covariates. Adjustment of results by survey sample weights and design was not reported. Only Blacks had significant Pb-systolic blood pressure associations; each doubling in blood Pb was associated with a 0.90 mm Hg (95% CI: 0.04, 1.8) and 1.20 mm Hg (95% CI: 0.4, 2.0) increase in males and females respectively. The association of Pb-diastolic blood pressure was also significant for Black females (0.50 mm Hg [95% CI: 0.01, 1.1]). Interestingly, increasing blood Pb was associated with significantly decreased diastolic blood pressure in White males (−0.6 mm Hg [95% CI: −0.9, −0.3]). The authors did not comment on their finding that the significant total serum calcium covariate in these two groups had opposite signs too (White male serum calcium $\beta = 6.50$ mm Hg/mmol/L, Black female serum calcium $\beta = -5.58$ mm Hg/mmol/L). Though the authors offered no formal test of the difference between the two serum calcium coefficients, since both were significantly different than the null hypothesis coefficient of 0 and different in sign, it could be concluded that those coefficients were significantly different between the two groups. As the authors do not present the serum calcium coefficients before forcing Pb into the models, it is not certain that blood Pb in the model was associated with the significant sign difference of the calcium coefficients or if the calcium coefficients had opposite signs between the two groups without Pb in the model. As each model had a different set of covariates, the presence or absence of one of the other covariates could have produced the same results. Still, this pattern of results may indicate significant confounding between serum calcium and blood Pb associations with blood pressure. Though the study suggested differences between Blacks and Whites in response to Pb, no statistical tests were performed of differences between Pb coefficients based on race. In addition, the Black-White effect differences associated with blood Pb may be due to possible confounding in some or all of the models.

Limiting the study sample from NHANES III to women aged 40 to 59 years, another group of researchers addressed the relationship between blood Pb and both blood pressure ($n = 1{,}786$) and hypertension ($n = 2{,}165$) over a blood-Pb range of 0.5 to 31.1 µg/dL (mean 2.9 µg/dL) (Nash et al., 2003). Blood pressure models excluded women who reported being under treatment for hypertension. Separate blood pressure multiple regression models were presented for diastolic and systolic blood pressure, each with and without stratification for

dichotomous premenopausal/postmenopausal status. One block of covariates was entered without regard to statistical significance (age, race/ethnicity, BMI, and serum creatinine). Another block of covariates (education, poverty income ratio, alcohol use, and cigarette smoking status) was entered second but only retained if variables were significantly associated with blood pressure. Finally, linear blood Pb was forced in last. Logistic regression models for hypertension used the same covariate entry scheme with and without stratification on the menopause variable, but using a blood Pb quartile exposure variable. Despite the stated procedure for covariate selection, all models used the same set of covariates: linear (or quartile) Pb, age, race/ethnicity, alcohol use, cigarette smoking status, BMI, and serum creatinine. All models were adjusted for survey weights and design. Linear Pb was significantly associated with systolic blood pressure only in the entire study sample; each 1 μg/dL increase in blood Pb was associated with a 0.32 mm Hg (95% CI: 0.01, 0.63) increase in blood pressure. No associations were observed in the menopause-stratified analyses. Linear Pb was also significantly associated with diastolic blood pressure in the entire study sample (0.25 mm Hg [95% CI: 0.07, 0.43]). Odd ratios of diastolic hypertension (>90 mm Hg) in logistic regression models were significantly related to blood Pb, with an odds ratio of 4.26 (95% CI: 1.36, 12.99) comparing the 1st quartile blood Pb group (0.5-1.6 μg/dL) to the 4th quartile blood Pb group (4.0-31.1 μg/dL) in all women not taking antihypertensive medications. Further stratification produced occasional significant odds ratios for either diastolic or systolic hypertension. There were some differences in table and text reporting of results and an inconsistency between the SE and the p-values.

Another study using the NHANES III database was notable for its formal testing of race and sex differences in Pb effect by interactions terms (Vupputuri et al., 2003). The study used 5,360 White men (mean blood Pb 4.4 μg/dL), 2,104 Black men (mean blood Pb 5.4 μg/dL), 5,188 White women (mean blood Pb 3.0 μg/dL), and 2,300 Black women (mean blood Pb 3.4 μg/dL). Blood Pb ranges were not given. Multiple linear and logistic regression models of blood pressure and hypertension (systolic ≥140 mm Hg, diastolic ≥90 mm Hg, and/or taking antihypertensive medication), respectively, were adjusted for age, high school education, BMI, alcohol, leisure-time physical activity, and dietary intake of sodium, potassium, and total energy. The models used linear blood Pb, except for one set of hypertension models with a cut point for "high" Pb exposure at ≥5 μg/dL. Subjects taking antihypertensive medication (n = 2,496) were

not included in linear regression models of blood pressure. Neither age nor blood Pb range were reported, nor was the technique of selecting and entering covariates in multiple regression models. Only coefficients for linear Pb effect for each model were reported. Significant interactions in multivariate models were found between Pb and race and between Pb and sex, though these analyses were not shown. Only Black men and women had significant linear Pb-blood pressure effects in adjusted systolic (0.25 mm Hg [95% CI: 0.06, 0.44] for Black men and 0.47 mm Hg [95% CI: 0.14, 0.80] for Black women with each 1 µg/dL increase in blood Pb) and diastolic blood pressure (0.19 mm Hg [95% CI: 0.02, 0.36] for Black men and 0.32 mm Hg [95% CI: 0.11, 0.54] for Black women). Linear blood Pb association with hypertension was significant only in women. The odds ratios were 1.09 (95% CI: 1.04, 1.13) for White women and 1.10 (95% CI: 1.06, 1.16) for Black women for each 1 µg/dL increase in blood Pb. The authors presented insufficient detail to evaluate this pattern of results.

6.5.2.2.2 Other U.S. Cohort Studies

The Boston-based Normative Aging Study, part of a longitudinal study of male veterans, examined the effects of blood Pb on blood pressure in 798 men, aged 45-93 years old, with blood Pb between 0.5 and 35.0 µg/dL (Proctor et al., 1996). Using multiple regression modeling with forced entry of natural log Pb and other covariates (age, age^2, BMI, dietary calcium, exercise, smoking, alcohol, heart rate, and hematocrit), the authors found a significant increase of only diastolic blood pressure (0.83 mm Hg [95% CI: 0.08, 1.52]) for each doubling of blood Pb. Though the relationship between blood Pb and systolic blood pressure was positive, it was not significant. Nearly half the blood Pb measures were derived from frozen red blood cells collected previously (up to several years earlier) and corrected for hematocrit determined at the time blood pressure was measured. Possible errors in correction of these samples and the non-contemporaneous nature of the resulting blood Pb concentrations may have compromised the results.

Cheng et al. (2001), using the same Normative Aging Study data and stepwise multiple regression, found a near-zero association between systolic blood pressure and linear blood Pb (−0.03 mm Hg for each µg/dL increase in blood Pb) in 519 men aged 48 to 93. The subjects selected for this analysis were all free of hypertension (systolic > 160 mm Hg or diastolic > 95 mm Hg). Differences in subject selection procedures and modeling techniques may have

accounted for the different results between Cheng et al. and Proctor et al. They also reported on incidence of hypertension developing between 1991 and 1997 using Cox proportional hazards models. Controlling for age, age^2, BMI, and family history of hypertension, linear blood Pb was not significantly associated with risk of developing hypertension (systolic > 140 mm Hg or diastolic > 90 mm Hg) in subjects normotensive at the start of the period (rate ratio of 0.98 [95% CI: 0.91, 1.06]) for each 1 µg/dL increase in blood Pb.

Gerr et al. (2002) similarly reported near-zero linear blood Pb effects on blood pressure on a combined group of 19-29 year old males and females (n = 502), half of whom had lived around active Pb smelters as children, using forced entry of all covariates. Mean blood Pb was 2.2 µg/dL with a range from <1 to >7 µg/dL. Among the covariates forced into the model was tibia Pb concentration, expected to be significantly correlated with blood Pb. This may have reduced or confounded the effects of blood Pb.

Korrick et al. (1999) examined linear and natural log blood Pb, mean 3 µg/dL (range <1 to 14 µg/dL), effects on hypertension, defined as self-reported or physician hypertension diagnosis (i.e., systolic or diastolic blood pressure ≥140/90 mm Hg), in 284 middle-aged women from the Nurse Health Study based in Boston. The association of hypertension and blood Pb was not significant. The study had low power (n = 284).

Rothenberg et al. (1999) tested a group of 1,527 women, aged 15 to 42 years, in their third trimester of pregnancy, with blood Pb ranging from 0.5 to 40.4 µg/dL. They stratified testing into immigrant (n = 1,188) and nonimmigrant (n = 439) groups. They used forced entry of all covariates in multiple regression models, including natural log Pb, age, BMI, coffee, iron supplement, and job stress, and found Pb-related significant increases in systolic (1.18 mm Hg [95% CI: 0.45, 1.91] for each doubling of blood Pb) and diastolic (1.02 mm Hg [95% CI: 0.37, 1.34]) blood pressure only in immigrants. The small size of the nonimmigrant group may have reduced power to detect significant effects. In a follow-up study of 668 women returning for postpartum testing (Rothenberg, et al., 2002a), using multiple regression models with forced entry of natural log blood Pb, tibia and calcaneus Pb, age, BMI, parity, smoking, immigrant status, and education, the authors found significant <u>decreases</u> in systolic (−1.05 mm Hg [95% CI: −1.96, −0.14]) and diastolic (−1.16 mm Hg [95% CI: −1.98, −0.35]) blood pressure associated with doubling in blood Pb in the postpartum women. This subgroup of women had no significant blood Pb effects in the third trimester. Although the covariate pattern was different

from the larger prenatal study (Rothenberg et al., 1999), thorough testing of possible confounding, especially with the bone Pb measures, revealed no significant change in blood Pb effects. This study finding is similar to that reported by Den Hond et al. (2002) for White males. No significant effect of blood Pb on prenatal or postpartum hypertension (≥140/90 mm Hg) was found.

Morris et al. (1990) recruited a group of 105 women and 145 men, aged 18-80 years, from a clinic specializing in nondrug hypertension treatment. Blood Pb ranged from 5 to 40.5 µg/dL. Multiple regression was performed with forced entry of natural log Pb, age, BMI, dietary calcium, "other nutrients," serum ionized calcium, and erythrocyte protoporphyrin. Only men were found to have blood Pb-related significant increases in systolic (3.17 mm Hg [95% CI: −2.13, 8.48] for each doubling of blood Pb) and diastolic (1.32 mm Hg [95% CI: −2.12, 4.75]) blood pressure. Small study size limits conclusions based on nonsignificant findings in women. Dietary calcium is associated with reduced blood Pb in many studies and could be considered a confounder with blood Pb. Erythrocyte protoporphyrin is a biomarker of Pb exposure and correlates with blood Pb over at least part of the blood range in study subjects. There were at least two variables collinear with blood Pb, a high proportion of covariates to subjects, and possible subject selection bias.

6.5.2.2.3 European Cohort Studies

The Glostrup Population Study (Copenhagen) evaluted data for 1,009 men and women (all born in 1936) longitudinally studied from 1976 to 1987 (Møller and Kristensen, 1992). Blood Pb levels ranged from 2 to 62 µg/dL, depending on the year and sex stratum studied, with mean concentration dropping by ~40% over the study period. Multiple regression analyses were used, with forced entry of natural log Pb, BMI, tobacco use, and physical activity. Strongest associations between a doubling of blood Pb and blood pressure were found early in the study period. In 1976, a doubling of blood Pb was associated with a 3.42 mm Hg (95% CI: 1.25, 5.58) increase in systolic blood pressure and a 2.95 mm Hg (95% CI: 1.08, 4.83) increase in diastolic blood pressure in women. For men in 1981, a doubling of blood Pb was associated with an increase of 1.89 mm Hg (95% CI: 0.00, 3.78) in systolic blood pressure and 1.14 mm Hg (95% CI: −0.37, 2.65) in diastolic blood pressure. No formal longitudinal analyses were performed, only analyses stratified by year and sex and analyses relating change in Pb and other covariates

to change in blood pressure from one study period to the next. As the relative risk of mortality was associated with increasing blood Pb over the study period (see below), the observed general reduction in the Pb-associated blood pressure increase over the study period may have been related to Pb-associated mortality.

The Europe New Risk Factor Project in Rome collected data from 1,319 males aged 55 to 75 years with blood Pb between 4.0 and 44.2 µg/dL (Menditto et al., 1994). They reported significantly increased systolic (4.71 mm Hg [95% CI: 2.81, 6.61]) and diastolic (1.25 mm Hg [95% CI: 0.33, 2.16]) blood pressure associated with a doubling of blood Pb.

The Cadmibel studies from Belgium specifically selected part of their study group from those living near nonferrous smelters. Staessen et al. (1993) reported on 827 men and 821 women, aged 20 to 88 years, with blood Pb ranging from 2.7 to 84.9 µg/dL for men and 1.3 to 42.4 µg/dL for women. They forced natural log blood Pb into stepwise multiple regression models stratified by sex. Covariates available for selection were age, age^2, BMI, pulse rate, log gamma-glutamlytranspeptidase, serum total calcium, log serum creatinine, urinary potassium, smoking, alcohol, contraceptive use, and menopause. Near-zero nonsignificant relationships were found between blood Pb and blood pressure for systolic blood pressure for women and diastolic blood pressure for men and women. They reported a significant decrease in men's systolic blood pressure with increasing blood Pb (−1.1 mm Hg for a doubling of blood Pb), similar to the relationship found by Den Hond et al. (2002) for White men and by Rothenberg et al. (2002a) for postpartum women. Stepwise regression results in different covariate patterns for each stratum and capitalizes on chance significance due to multiple testing.

In a follow-up of the Cadmibel study, the PheeCad study evaluated 359 men and 369 women, aged 20 to 82 years (Staessen et al., 1996a). Fifty-nine percent of the men had occupational Pb exposure. They were measured twice, at baseline and at follow-up about 5 years later. Men's mean blood Pb at baseline and follow-up was 11.4 µg/dL (range 5.6-28.8) and 7.7 µg/dL (range 3.7 to 20.1). Women's mean blood Pb at baseline and follow-up was 6.6 µg/dL (range 3.3-24.50 and 4.8 µg/dL (range 1.7-11.8). Multiple regression models were stratified on sex and, in women, on menopausal status. Time-integrated blood pressure measurements were used. Each doubling of log blood Pb was significantly associated with a 5.19 mm Hg (95% CI: 1.05, 9.34) increase in diastolic blood pressure in 187 pre- and perimenopausal women. None of the other strata showed significant blood Pb-related effects. Using 24-h ambulatory blood

pressure readings during the follow-up showed significant associations between natural log blood Pb and diastolic blood pressure in the group of all 345 women (2.42 mm Hg [95% CI: 0.00, 4.84]). There were no significant Pb effects on systolic blood pressure in women or all blood pressure in men. Change in blood pressure and change in covariates between baseline and follow-up were used to assess the effect of change of blood Pb in longitudinal analyses, similar to Møller and Kristensen (1992) above. No significant effects of change in blood Pb on change in blood pressure were found. Due to stratification and resulting small groups, there may have been reduced power to detect significant Pb effects.

The Health Survey for England 1995 examined a representative sample of the English population living in private households and provided up to 2,563 men and 2,763 women with a mean age of 47.6 years in a study of blood Pb-blood pressure relationships (Bost et al., 1999). Precise blood Pb ranges were not given, but were at least from less than 1.5 µg/dL to greater than 8.5 µg/dL, with geometric means of 2.6 µg/dL (females) and 3.7 µg/dL (males). The study used stepwise multiple regression modeling of diastolic and systolic blood pressure stratified by sex, with and without adjustment for alcohol, and with and without subjects on antihypertensive medications. Candidate covariates, selected from a larger pool, included age, alcohol use (heavy drinkers versus all other drinkers and nondrinkers), SES (manual classes versus non-manual classes), location of residence in country (northern resident versus non-northern resident), smoking, and common log blood Pb. As nonsignificant variables did not remain in the models, each model contained a unique mix of covariates. A doubling in blood Pb in men was associated with an increase in diastolic blood pressure of 1.07 mm Hg (95% CI: 0.37, 1.78) when alcohol consumption was not in the model and 0.88 mm Hg (95% CI: 0.13, 1.63) when alcohol consumption was in the model. Women had a significant response to Pb only for diastolic blood pressure in the model without adjustment for alcohol and with subjects using antihypertensive medication. There were no significant Pb effects on systolic blood pressure in any model. The authors provided no statistical justification for stratified modeling nor did they test for significant differences in Pb coefficients as a result of the stratification.

6.5.2.2.4 Occupational Studies

U.S. Occupational Studies

Glenn et al. (2003) was one of the few studies to use a prospective design and was the only study using statistical techniques designed for repeated measures. They studied 496 male workers from New Jersey with former organolead exposure. Using generalized estimating equations (GEE) with baseline linear blood Pb, age, BMI, smoking, education, antihypertensive medication, measurement technician, and number of years to follow-up measurement of blood pressure (range 10 months-3.5 years), they found every 1 μg/dL increase in baseline blood Pb to be associated with 1.13 mm Hg/year (95% CI: 0.25, 2.02) increase in blood pressure over the observation period.

Schwartz et al. (2000c) reported significant blood Pb associations with 543 male former organolead workers. Stepwise backward multiple regression showed an increase of 2.3 mm Hg in systolic blood pressure for each doubling in blood Pb. The association with diastolic blood pressure was not significant.

Sharp et al. (1990) studied 132 Black bus drivers (blood Pb range 3.1-20.9 μg/dL) and 117 non-Black bus drivers (blood Pb range 2.0 to 14.7 μg/dL) in San Francisco, aged 30 to 60 years. They used natural log blood Pb in multiple regression models and found for each doubling of blood Pb an increase of 5.22 mm Hg (95% CI: 0.60, 9.84) in systolic blood pressure among Blacks, 3.27 mm Hg (95% CI: 0.10, 6.44) in diastolic blood pressure among Blacks, and −3.96 mm Hg (95% CI: −8.32, 0.42) in systolic blood pressure among non-Blacks.

Sokas et al. (1997) reported a possible race interaction ($p = 0.09$) on systolic blood pressure with linear blood Pb in 264 construction workers aged 18-79 years. Each 1 μg/dL increase in blood Pb increased systolic blood pressure in Blacks by 0.86 mm Hg more than in Whites. Neither the Black or White Pb coefficients were significant.

European Occupational Studies

Maheswaran et al. (1993) reported on 809 male factory workers with blood Pb levels between <21 to >50 μg/dL from Birmingham, England. Unfortunately, the inclusion of other factors strongly related to blood Pb, including an additional direct measure of Pb exposure (years working in factory) in addition to linear blood Pb and inclusion of zinc protoporphyrin, may have biased the blood Pb effect and resulted in nonsignificant Pb effects on blood pressure.

Telišman et al. (2004) also reported nonsignificant effects of natural log blood Pb on blood pressure in 115 male industrial workers with blood Pb levels between 9.9 and 69.9 µg/dL, but included erythrocyte protoporphyrin in models, a variable correlated with blood Pb over much of the observed blood Pb range. Coefficients were not given, as Pb did not enter into stepwise regression models. The study had very low power.

Asian Occupational Studies

Male and female factory workers (n = 798) from Chonan, Korea (blood Pb between 17.8 and 64.8 µg/dL) were studied principally for the effects of genotype of ALAD and vitamin D receptor on cardiovascular response to Pb (Lee et al., 2001). These aspects are covered more thoroughly below. As part of their work, the authors developed multiple regression models examining the effect of linear blood Pb on blood pressure with forced entry of age and age^2, BMI, sex, antihypertensive medication, lifetime alcohol, and ALAD and vitamin D genotypes. A marginally significant effect of blood Pb on systolic blood pressure (diastolic blood pressure not modeled) was noted, with a 10 µg/dL increase in blood Pb associated with a 0.7 mm Hg (95% CI: −0.04, 1.4) increase in blood pressure.

Nomiyama et al. (2002) used a combined group of 193 female crystal glass workers and nonexposed controls, aged 16 to 58 years, with blood Pb between 3.8 and 99.4 µg/dL. The authors used a stepwise multiple regression with a novel technique to reduce collinearity among covariates. From a large group of covariates, they selected covariates eligible to enter the regression from a factor analysis. Although the stepwise entry of these variables resulted in different models for systolic and diastolic blood pressure, both models included linear blood Pb, age, urine protein, and plasma triglycerides. The diastolic model additionally included family hypertension and low density lipoprotein. Each 10 µg/dL increase in blood Pb was significantly associated with a 1.26 mm Hg (95% CI: 0.58, 1.94) increase in systolic blood pressure and a 1.05 mm Hg (95% CI: 0.52, 1.57) in diastolic blood pressure. In alternative models with ordered categories of blood Pb, systolic blood pressure was 7.5 mm Hg (95% CI: 3.0, 12.0) and diastolic blood pressure was 6.3 mm Hg (95% CI: 3.4, 9.1) higher in workers with blood Pb ≥60 µg/dL than in controls with <11.4 µg/dL. Models did not control for BMI.

Wu et al. (1996) examined the effect of ordered blood Pb category on blood pressure of 112 male (aged 18-67 years) and 110 female (aged 18-71 years) Pb battery factory workers in

multiple regression models. Blood Pb ranged from 8.3 to 95.4 µg/dL. Nonsignificant blood Pb effects were found possibly due to the inclusion of two additional Pb exposure measurements, ambient air Pb and work history, likely leading to substantial collinearity with blood Pb.

6.5.2.2.5 Meta-Analyses of Blood Lead-Blood Pressure Studies

The most recent meta-analysis of the blood Pb-blood pressure literature analyzed 31 studies from a large pool of studies published up to 2001 (Nawrot et al., 2002). Two other meta-analyses (Schwartz, 1995; Staessen et al., 1994) that were also published during this reporting period covered many of the earlier papers cited in Nawrot et al. (2002) and derived similar coefficients for the Pb effect; so, they are not reviewed here. The Nawrot et al. (2002) meta-analysis authors selected studies with 50 or more subjects, with subjects 10 years of age and up, with blood pressure and blood Pb measurement techniques presented in sufficient detail to estimate effect sizes, and with preference given to papers with models adjusting for age, BMI, and "additional factors of proven importance." Where possible, studies with stratified analyses based on sex and race were entered in the meta-analysis as separate subgroups. Studies were weighted by the number of subjects to arrive at estimates and CIs for Pb effect on diastolic and systolic blood pressure. Nearly half the studies reported Pb effects from linear Pb terms, the remainder from log-transformed Pb. To include both types of studies in the analyses, the authors reported effect sizes based on doubling the mean blood Pb concentration. For models using logarithmic blood Pb, this doubling has the same effect anywhere in the range of blood Pb in the study. For models using linear blood Pb, the doubling effect was referenced from the mean blood Pb reported. Figures 6-10 and 6-11 depict the effect estimates for systolic and diastolic blood pressure, respectively, included in the meta-analysis from Nawrot et al. (2002). Ninety-five percent CIs overlapped for males and females and for Blacks and Whites, suggesting to Nawrot et al. no significant differences in Pb effect by gender or race. The results from the various studies are generally consistent, with a large majority indicating positive effects. In the group of studies as a whole, the combined meta-analysis coefficients for each doubling of blood Pb were highly significant for both systolic (1.0 mm Hg [95% CI: 0.5, 1.4]) and diastolic (0.6 mm Hg [95% CI: 0.4, 0.8]) blood pressure. The meta-analysis provides strong evidence for an association between increased blood Pb and increased blood pressure over a wide range of populations.

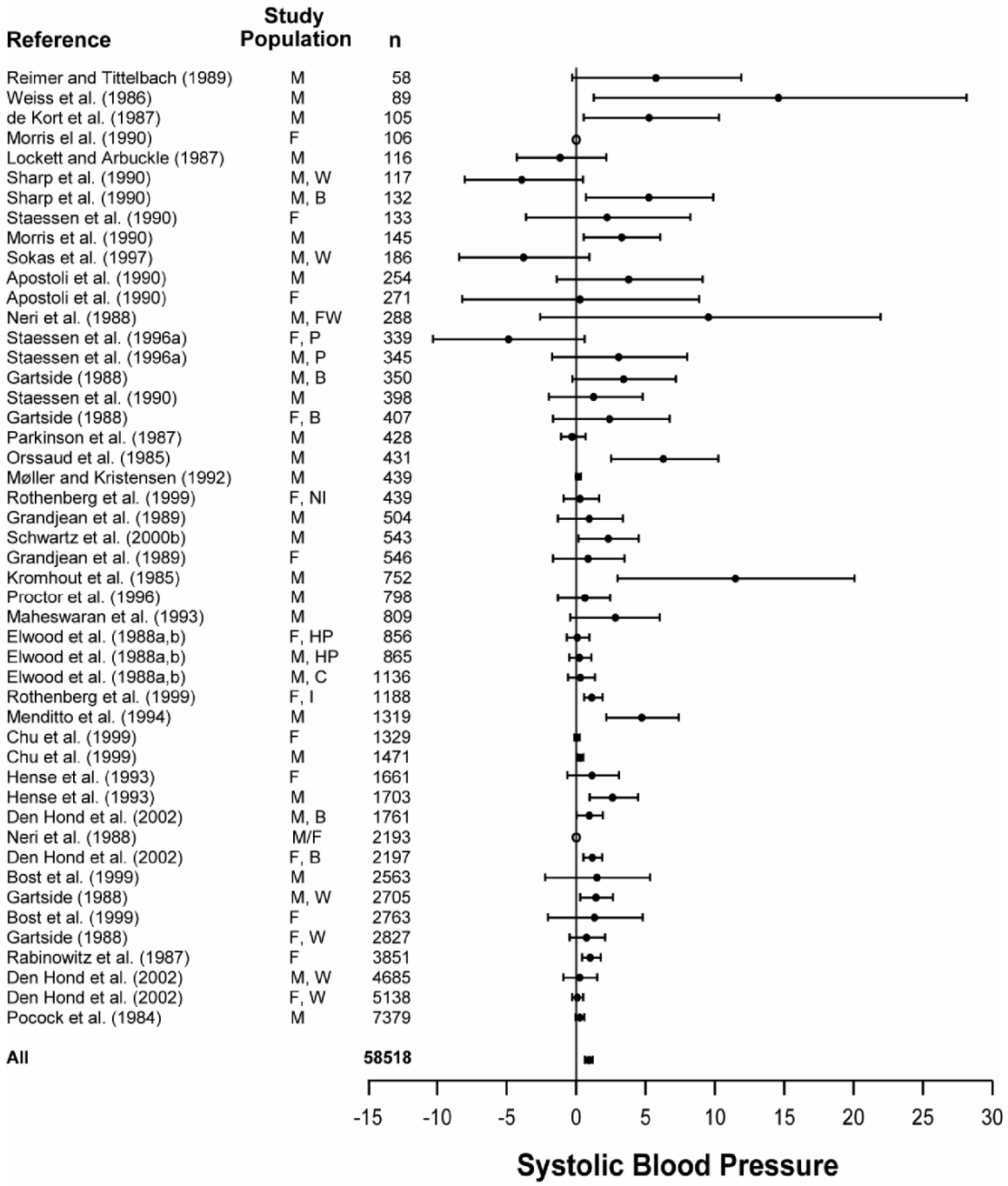

Figure 6-10. Change in the systolic pressure (effect estimate in mm Hg) associated with a doubling of the blood lead concentration. Studies arranged vertically by increasing study size.

Study key: C = Caerphilly Study, HP = Welsh Heart Program, P = PheeCad Study, W = Whites, B = Blacks, NI = nonimmigrants, I = immigrants, FW = foundry workers, CS = civil servants.

Source: Nawrot et al. (2002).

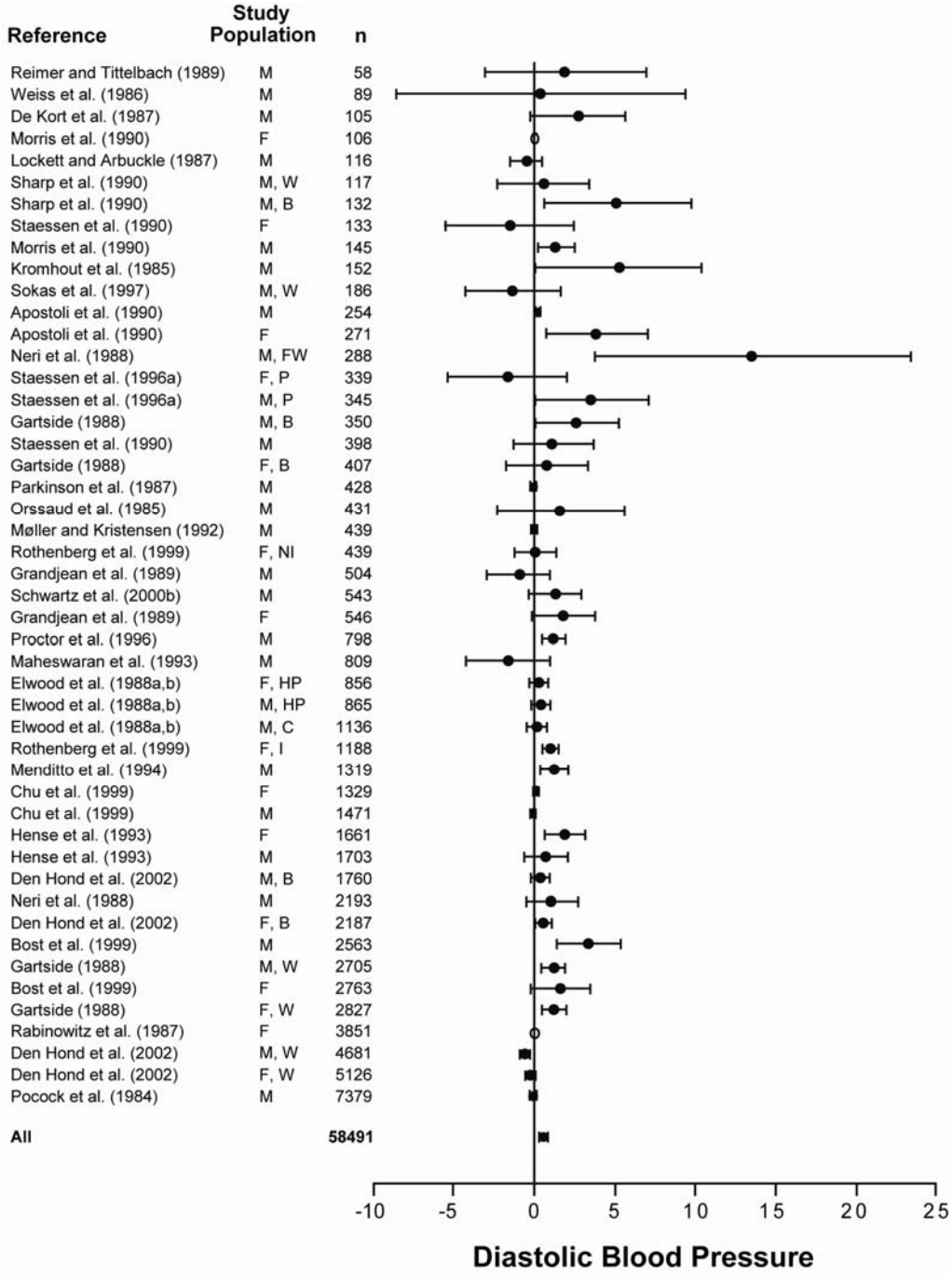

Figure 6-11. Change in the diastolic pressure (effect estimate in mm Hg) associated with a doubling of the blood lead concentration. Studies arranged vertically by increasing study size.

Study key: C = Caerphilly Study, HP = Welsh Heart Program, P = PheeCad Study, W = Whites, B = Blacks, NI = nonimmigrants, I = immigrants, FW = foundry workers, CS = civil servants.

Source: Nawrot et al. (2002).

Figure 6-12 compared the effect estimates obtained from studies using log-linear versus linear models. Several of the studies from Nawrot et al. (2002) are included. The studies shown in Figure 6-12 were selected to include those studies with subjects having contemporary blood Pb with contemporary blood pressure, published 1990 to present. Effects for the entire study population are presented unless only effects in subsamples are reported. Other selection criteria used are detailed in the legend of Figure 6-12. Results from these individual studies also generally appear to agree with the results of the meta-analysis by Nawrot et al. that increased blood Pb levels are significantly associated with increased systolic and diastolic blood pressure. A random effects meta-analysis was preformed to examine the use of log-linear and linear blood Pb models in blood pressure studies. A significant blood Pb effect on systolic blood pressure was observed for both the log-linear ($p = 0.05$) and linear models ($p < 0.001$). Heterogeneity was significant for the log-linear model ($p = 0.0002$), but not the linear model ($p = 0.319$). The log-linear and linear effects were 0.62 mm Hg (95% CI: 0.12, 1.11) and 0.55 mm Hg (95% CI: 0.33, 0.772) per 5 μg/dL respectively, for systolic blood pressure. The difference between these effect estimates using linear or log linear models is non-significant. A meta-regression analysis was done using the geometric mean of the blood Pb in each study. Geometric mean blood Pb was insignificant, indicating that the heterogeneity found is not due to the slopes varying with the mean level of blood Pb. These meta-analyses suggest there may be some differences between the studies, but overall there is an effect of blood Pb on systolic blood pressure. Furthermore, the meta-analyses results suggest that studies not detecting an effect may be due to small sample sizes or other factors affecting precision of estimation of the exposure-effect relationship.

6.5.2.3 Blood Pressure and Hypertension Studies Using Bone Lead as Exposure Index

Since the 1990 Supplement, several studies have examined the association between Pb and blood pressure or hypertension using bone Pb levels as the exposure index. The key studies are discussed here, and additional studies are summarized in Annex Table AX6-5.1.

Korrick et al. (1999) used a case-control design to study relationships between hypertension in women and three measures of Pb exposure: blood Pb, tibia (cortical bone) Pb, and patella (trabecular bone) Pb. The final study sample consisted of 89 hypertension cases and

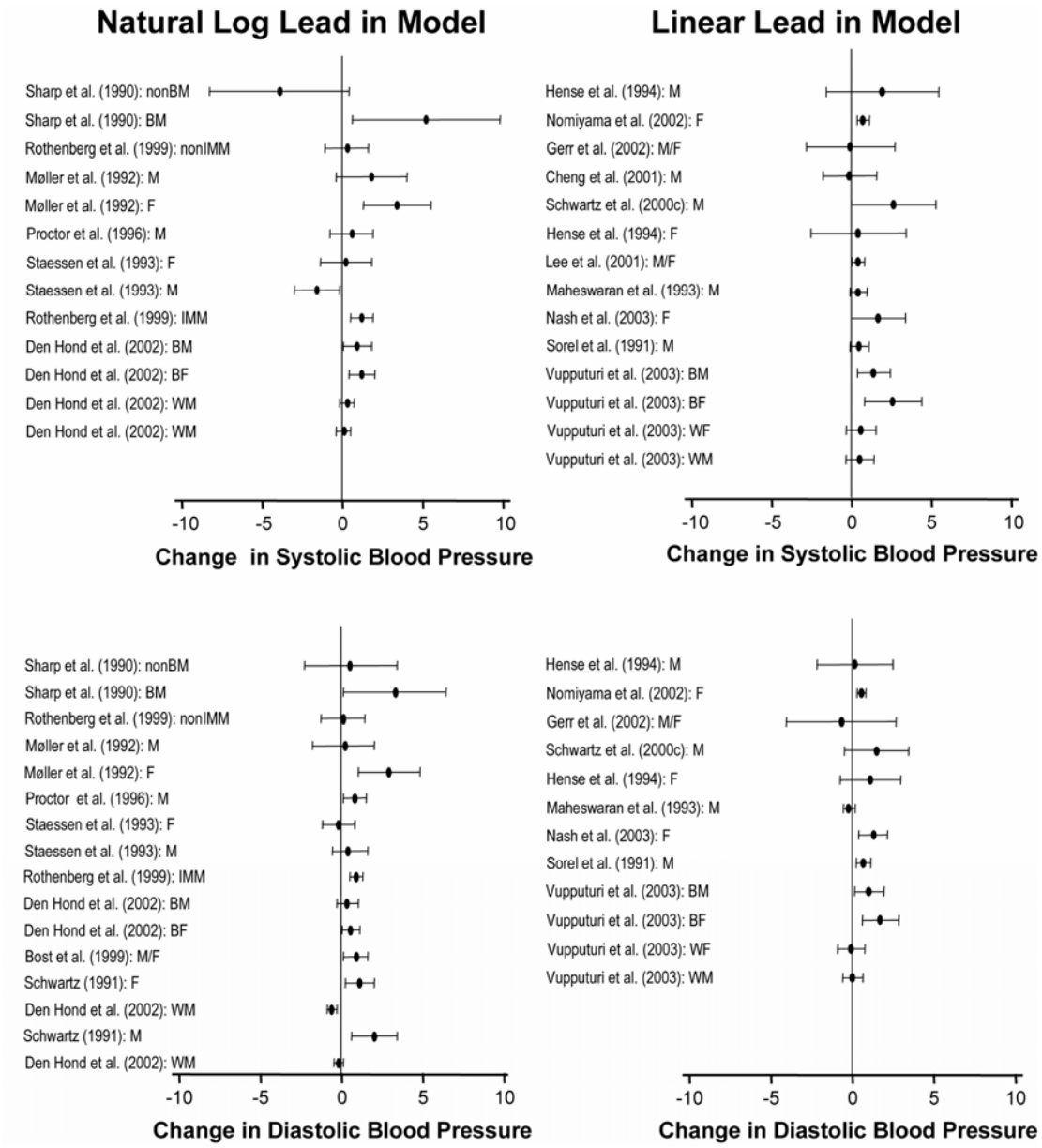

Figure 6-12. Effect of doubling mean blood lead on estimate of blood pressure change with 95% CIs. In studies using linear blood lead terms the effect size was calculated using blood lead doubling from 5 to 10 µg/dL. Studies not reporting sufficient information to present coefficients and CIs were not included. Studies arranged vertically by increasing study size. Where multiple models from the same study were presented, such as repeated measures over time or adding a confounding variable, only the effect estimate from the first model is shown. When the same study was multiply published with subsamples, only the effect estimate from largest study is shown.

Study key: B = Blacks, W = Whites, M = males, F = females, IMM = immigrants, nonIMM = nonimmigrants.

195 controls, excluding those with history of hypertension, cardiovascular disease, renal disease, diabetes, or malignancy, use of antihypertensive medications, BMI ≥29, and incomplete data, aged from 47 to 74 years. Cases were selected through a randomization procedure that produced approximately equal numbers of cases for each of three blood pressure categories, hypertensive (≥140 mm Hg or 90 mm Hg), high normal (≥121/75 mm Hg up to hypertension limit), and low normal (<121/75 mm Hg). As many as four controls were matched to cases by 5 year age grouping. Though they did not match cases and controls on other potential confounding variables, they included these variables in their models. The dependent variable was constructed by placing blood pressure measurements into the three groups. The mean blood Pb level was 3.1 µg/dL; the mean tibia and patella Pb levels were 13.3 µg/g and 17.3 µg/g, respectively. An ordered logistic regression with proportional odds assumptions was used to asses linear blood Pb, patella and tibia bone Pb effects on odds of hypertension, controlling for age, BMI, dietary calcium, alcohol use, dietary sodium, smoking, and family hypertension. They presented results from four models with the same covariates determined a priori, but with each Pb variable tested separately. Only patella Pb concentration significantly ($p = 0.03$) predicted increased odds for hypertension, but the effect was small. Each 10 µg/g increase in patella Pb was associated with an odds ratio of 1.28 (95% CI: 1.03, 1.60). Separate analyses testing interactions of alcohol use, age, and menopausal status showed no significant interaction with patella Pb, though the small sample size had little power to detect significant interaction effects. Model diagnostics were given for justifying the use of proportional odds ordinal regression, but none were given justifying use of a linear blood Pb term in the models.

Rothenberg et al. (2002a) investigated associations between both hypertension and blood pressure with blood Pb, tibia Pb, and calcaneus Pb in 668 women, aged 15 to 44 years, in the third trimester of pregnancy and during a 3-month postpartum period using a cohort design and multiple logistic and multiple linear regression modeling. Subject exclusion criteria were blood Pb > than 5 geometric SDs from the geometric mean, documented renal disease, cardiovascular disease, diabetes, use of stimulant drugs, and extreme postnatal obesity (BMI >40). Geometric mean prenatal and postnatal blood Pb levels were 1.9 µg/dL and 2.3 µg/dL, respectively. Mean tibia and calcaneus Pb levels were 8.0 µg/g and 10.7 µg/g, respectively. Variables in all models were selected a priori and retained in the models regardless of significance level. Control variables were education, smoking status, immigrant status, parity, age, and BMI in all models.

Prenatal models also controlled for postpartum hypertension in lieu of family history of hypertension. None of the subjects used antihypertensive medications during the study. All three Pb variables were simultaneously tested in all models. Third trimester blood Pb ranged from 0.4 to 30.0 µg/dL, postpartum blood Pb ranged from 0.2 to 25.4 µg/dL. Calcaneus Pb ranged from −30.6 to 49.9 µg/g and tibia Pb ranged from −33.7 to 42.5 µg/g. Only calcaneus Pb was significantly associated with an increase in hypertension (either ≥140 mm Hg systolic or ≥90 mm Hg diastolic) during pregnancy, with an odds ratio of 1.86 (95% CI: 1.04, 3.32) for each 10 µg/g increase of calcaneus Pb. No association between calcaneus Pb and hypertension was found postpartum. The authors found the same pattern of trabecular Pb concentration association with blood pressure during but not after pregnancy in normotensive women. A 10 µg/g increase in calcaneus Pb was associated with ~0.75 mm Hg (95% CI: 0.04, 1.46) increase in systolic and ~0.58 mm Hg (95% CI: 0.01, 1.16) increase in diastolic blood pressure in the third trimester. Thorough diagnostic testing was performed for all models. Only linear age terms were used in the models without exploration of age^2 terms. The authors did not use the repeated measures nature of the design in their analyses; instead they analyzed third trimester pregnancy data and postpartum data separately. They did not statistically test differences in coefficients from the same variables in the two parts of the study.

Two studies examined a subset of subjects participating in the Normative Aging Study. Hu et al. (1996) used a cross-sectional design of 590 men with median age in the mid-60s (range 48-92 years). Blood Pb ranged from 1 to 28 µg/dL, tibia Pb from <1 to 96 µg/g, and patella Pb from 1 to 142 µg/g. Logistic regression models were initially constructed by adding age, race, BMI, family history of hypertension, smoking, alcohol use, and dietary sodium and calcium. Testing linear blood Pb, tibia Pb, and patella Pb one by one against hypertension status (systolic >160 mm Hg, diastolic >96 mm Hg, or taking antihypertensive medication), they found no significant relationships with any of the Pb variables, each entered separately. Only when they used backward elimination of nonsignificant variables did they find a significant odds ratio of 1.50 (95% CI: 1.09, 2.10) for each doubling of tibia Pb from the mean (20.8 µg/g) for hypertension.

Later, Cheng et al. (2001) followed up the same group, constructing a multiple linear regression model for systolic blood pressure (diastolic blood pressure was not mentioned in model descriptions) in subjects not hypertensive at baseline measurement. They used a fixed set

of control variables, including age and age terms, BMI, family history of hypertension, and alcohol and calcium intake, selected by univariate and bivariate testing of a larger set. After entering linear blood Pb, tibia Pb, and patella bone Pb separately into the models, they reported a significant association only with tibia Pb (1.60 mm Hg [95% CI: 0.00, 4.44] increase in systolic blood pressure for each doubling of tibia Pb from the mean). Several years later (not specified in methods but no more than 6 years), the group of subjects that was originally not classified as having definite hypertension was retested for presence of definite hypertension (≥160/95 mm Hg). Each Pb measure was separately entered into a Cox's proportional hazards model of incident definite hypertension. Only patella Pb showed a significant increase in the rate ratio in subjects with no history of definite hypertension, 1.14 (95% CI: 1.02, 1.28) for each 10 µg/g increase in patella Pb. Similar results were obtained when the borderline hypertensive group (>140/90 mm Hg) was combined with the definite hypertension group in patella Pb. A rate ratio of 1.23 [95% CI: 1.03, 1.48]) was estimated. Use of linear Pb terms may have affected the ability of the studies to detect significant blood Pb effects.

A pair of studies, using the same group of male workers (age range 42 to 74 years) previously exposed to organic and inorganic Pb at an industrial plant in the United States, investigated the role of blood Pb and bone Pb on blood pressure. Blood Pb ranged between 1 and 20 µg/dL, and tibia Pb from −1.6 to 52 µg/g. The study by Schwartz et al. (2000c) controlled for age, BMI, current smoking, and current use of antihypertensive medication in backward elimination linear multiple regression models for blood Pb, tibia Pb, and DMSA-chelatable Pb, forcing each Pb term into separate models. Only blood Pb was a significant predictor of blood pressure. In multiple logistic regression models, only blood Pb in workers <58 years of age was significant in predicting hypertension (>160/96 mm Hg). Although this study used linear blood Pb in one model, it used another model with both linear and squared-blood Pb. Both Pb terms were significant in the respective models.

In a follow-up study (Glenn et al., 2003) with most of the same subjects from the first study, subsequent measurements of blood pressure occurred at intervals of 4-12 months for 10.2 months to 3.5 years. The study was notable not only for its prospective nature but in the use of statistical models adjusting for repeated measurements. Models were constructed by adding to a base model containing age at start of study, race, BMI, and indicator variables for technician. Lead variables were always forced in the models, but it is not clear if they were each tested

separately. Other potential confounder variables were added stepwise to the model if they met a probability criterion. Both increasing linear blood Pb and tibia Pb were significantly associated with increasing systolic blood pressure times the number of years of follow-up blood measurement, but not with change in diastolic blood pressure. Each 10 μg/g increase in tibia Pb was associated with a 0.78 mm Hg, year (95% CI: 0.24, 1.31) increase in systolic blood pressure for workers followed for the longest time.

Gerr et al. (2002) tested the effect of blood Pb and tibia Pb only in young adults (age 19-29 years), both males and females, on blood pressure. Half the subjects had grown up around an active Pb smelter. Multiple linear regression models always used age, sex, height, BMI, current smoking status, frequency of alcohol consumption, current use of birth-control medication, hemoglobin level, serum albumin, and income, regardless of significance levels. Both blood Pb (as a linear term) and bone Pb (a four category ordinal variable from <1 μg/g to >10 μg/g) were tested together. Tibia Pb concentration in the highest group was associated with a significant increase in both systolic (4.26 mm Hg) and diastolic (2.80 mm Hg) blood pressure when compared to the lowest tibia Pb group.

6.5.3 Other Cardiovascular Outcomes

Cardiovascular morbidity studies reviewed in this section are further summarized in Annex Table AX6-5.2. Cardiovascular mortality studies are presented in Annex Table AX6-5.3.

6.5.3.1 Ischemic Heart Disease

A community-based case-referent study taken from the Stockholm Heart Epidemiology Program compared survivors of first-time myocardial infarction with matched referents based on sex, age, year of study enrollment, and hospital catchment area (Gustavsson et al., 2001). The authors assessed Pb exposure by a three category ordinal scale based on Pb levels in airborne dust. In the comparison of unexposed to >0-0.03 mg/m^3 (mean 0.01 mg/m^3) and unexposed to >0.04 mg/m^3 (mean 0.10 mg/m^3), the relative risk was 0.88 (95% CI: 0.69, 1.12) and 1.03 (95% CI: 0.64, 1.65), respectively.

In a reanalysis of the NHANES II dataset, the influence of linear blood Pb on the diagnosis of left ventricular hypertrophy (LVH), based on examination of electrocardiograms and body habitus data in ~9,900 subjects (exact number not given) aged 25 to 74 years, was

tested in a survey-adjusted stepwise logistic regression model (Schwartz, 1991). The final model adjusted LVH by age, race, and sex. The odds ratio for LVH was 1.33 (95% CI: 1.20, 1.47) for each 10 µg/dL increase in blood Pb over an unreported blood Pb range. The author reported no significant interactions between blood Pb and race or between blood Pb and sex, though the article noted that the number of cases of LVH was small. The linear Pb effect had greater significance than the natural log Pb effect, the reverse of the relationship between the two Pb specifications usually seen when blood pressure is the outcome variable.

In another study of electrocardiograms in 775 men (mean age 68 years, range 48-93) from the Normative Aging Study, patella and tibia Pb concentrations were significantly associated with increased heart rate-corrected QT and QRS intervals in men under 65 years but not over 65 years old in multiple regression stepwise analysis (Cheng et al., 1998). Only tibia Pb concentration was significantly associated with an increased odds ratio of intraventricular conduction deficit (2.23 [95% CI: 1.28, 3.90]) for every 10 µg/g increase in tibia Pb), but only in men under 65 years. In contrast, both tibia and patella Pb concentration were significantly associated with atrioventricular conduction deficit (odds ratio of 1.22 [95% CI: 1.02, 1.47] and 1.14 [95% CI: 1.00, 1.29] for each 10 µg/g increase in tibia and patella Pb, respectively), but only for men ≥65 years old. None of the Pb measurements were significantly associated with arrhythmia. Linear blood Pb terms were not significantly associated with any of the above outcomes. Though the authors reported examining both saturated models (models with all considered control and confounding variables, significant or not) and stepwise models, only stepwise models were presented or discussed with each Pb term forced into separate models. Thus, each model had an individual mix of control/confounding variables, though age was common to all models. Despite using age as a control/confounding variable in all models, the article offered no statistical justification for the age-stratified analysis.

A group of male and female battery factory workers (n = 108) working for at least 10 years and who were hired from 1960 to 1983 had blood Pb levels during 1970 to 1994 that ranged from 5 to 93 µg/dL (Tepper et al., 2001). Using a fixed covariate multiple logistic regression model, including age, BMI, sex, and family history of hypertension, the authors found a nonsignificant odds ratios for risk of hypertension (>165/96 mm Hg or self-reported use of hypertension medications) comparing the first tertile (138-504 µg/dL·year) cumulative blood Pb index with the third tertile (747-1447 µg/dL·year) index. Echocardiogram left ventricular mass

was not significantly related to cumulative blood Pb index or time-weighted average blood Pb. The study had very low power to detect significant effects.

The discrepancy in blood Pb results between the two electrocardiogram studies by Schwartz (1991) and Cheng et al. (1998) could well be explained by population differences. Though both used large datasets, the age range of the NHANES II subject pool was between 25 and 74 years and used both men and women, whereas the age range for the Normative Aging study was 48 to 93 years and used only men. Furthermore, the Cheng et al. study had 775 subjects whereas the Schwartz had a much larger, though unspecified number. The Tepper et al. (2001) study had the least number of subjects (n = 108), which may have resulted in not detecting significant effects on a different measure of LVH. Still, both electrocardiogram studies reported a significant Pb effect, and the study with bone Pb (Cheng et al., 1998) is particularly interesting, not only for its older sample but because the bone Pb exposure measure reflected accumulated past exposure, which blood Pb only partly reflects. The two studies are in agreement that Pb exposure, either past or present, is significantly associated with ischemic heart disease.

6.5.3.2 Cardiovascular/Circulatory Mortality

A recent follow-up of the NHANES II cohort provided mortality data used to associate past blood Pb concentration with increased circulatory mortality in the U.S. population (Lustberg and Silbergeld, 2002). Blood Pb concentration as measured during 1976 to 1980 was divided into three categories (<10 µg/dL, 10-19 µg/dL, and 20-29 µg/dL) after eliminating 109 subjects with blood Pb ≥30 µg/dL, leaving 4,190 subjects 30-74 years of age in the mortality sample followed to the end of 1992. During the follow-up period, 929 subjects died of all causes. ICD-9 codes 390-459 (circulatory) accounted for 424 deaths. Proportional hazards models using a priori selected potential confounding variables (age, sex, race, education, income, smoking, BMI, exercise, and location) were used to calculate risk ratios of cardiovascular mortality for the two higher Pb categories compared to a <10 µg/dL reference. The 20-29 µg/dL category showed significantly elevated relative risk of 1.39 (95% CI: 1.01, 1.91) for cardiovascular mortality.

Although the NHANES II analysis using data from 1976 to 1980 suggested an increased risk of mortality at blood Pb levels above 20 µg/dL, blood Pb levels have dramatically decreased since the late 1970s. More recent data from NHANES have found that the geometric mean

blood Pb levels decreased from 12.8 µg/dL in 1976-1980 to 2.8 µg/dL in 1988-1991 (Annest et al., 1983) and 2.3 µg/dL in 1991-1994 (CDC, 1997). NHANES III data (1988-1994) were used to further analyze risk of mortality in adults (age 40 years) at lower blood Pb levels (Schober et al., 2006). A total of 9,757 subjects were followed for a median of 8.55 years during which there were 2,515 deaths. An increased risk of cardiovascular mortality was associated with blood Pb levels of 5-9 µg/dL and 10 µg/dL compared to 5 µg/dL. The relative risk was 1.20 [95% CI: 0.93, 1.55] for 5-9 µg/dL and 1.55 [95% CI: 1.16, 2.07] for 10 µg/dL, and the test for trend was statistically significant. Increased risks of all cause and cancer mortality also were observed at blood Pb levels of 5-9 µg/dL compared to <5 µg/dL (relative risk of 1.24 [95% CI: 1.05, 1.48] for all cause mortality and 1.44 [95% CI: 1.12, 1.86] for cancer mortality). The authors noted that an important limitation of this study was that exposure classification was based on one blood Pb level measurement taken at baseline. Older individuals were more likely to have notably higher past peak and cumulative Pb exposure, and their blood Pb levels might have been disproportionately influenced by release of Pb from bone stores compared to younger individuals.

Another longitudinal study combined fatal and nonfatal coronary heart disease (ICD-8 codes 410-414) and cardiovascular disease (ICD-8 codes 410-414 and 430-435) categories from a Danish 1936 birth cohort (n = 1,052) followed from 1976 to 1990 (Møller and Kristensen, 1992). During the study period, 54 cases of cardiovascular disease with 19 deaths were reported. Log-transformed blood Pb was used in a Cox proportional hazards model, controlling for a priori selected variables of tobacco use, cholesterol, physical activity, sex, systolic blood pressure, and alcohol. Two other models were also examined, those leaving out alcohol or both alcohol and systolic blood pressure. None of the adjusted models showed significant risk hazard for combined fatal and nonfatal cardiovascular disease, though blood Pb was significantly associated with outcome in all models except the one containing both alcohol and systolic blood pressure for "total mortality" risk hazard. This article is notable for its detailed discussion of using confounding variables, such as hemoglobin and alcohol use, in multivariate models of Pb-cardiovascular associations. However, small sample size and low death rate may have contributed to the nonsignificant results.

An occupational study, using 1,990 male workers who worked at least 1 day between 1940 and 1965 in an active Pb smelter in the United States (mean length of employment at

smelter 13.8 years; mean estimated length of Pb exposure 9.9 years), failed to show an association between Pb and standardized mortality ratios compared to the U.S. population reference group up to 1988 (Steenland et al., 1992). Neither mortality from ischemic heart disease (ICD-9 410-414), hypertension with heart disease (ICD-9 402 and 404), hypertension with no heart disease (ICD-9 401, 403, and 405), nor cerebrovascular disease (ICD-9 430-438) were significantly higher in the study group than in the U.S. population when examined in their totality or stratified by "high Pb exposure" (>0.2 mg/m^3 Pb in air, surveyed in 1975) or "duration of exposure." Imprecise estimation of Pb exposure may have contributed to the nonsignificant results.

A study of 664 male workers in a Swedish Pb smelter from 1942-1987 evaluated standardized mortality ratios for cardiovascular disease compared to the county population mortality figures from 1969-1989 (Gerhardsson et al., 1995). Blood Pb measurements were available from the workers since 1969 (mean 62.1 µg/dL) and dropped steadily from that date to 1985 (mean 33.1 µg/dL). The consecutive blood Pb measurements in the subjects allowed construction of a cumulative blood Pb index. Standardized mortality ratios were significantly elevated in the group for all cardiovascular diseases (ICD-8 390-458) and for ischemic heart disease (ICD-8 410-414), 1.46 (95% CI: 1.05, 2.02) and 1.72 (95% CI: 1.20, 2.42), respectively. However, there were no indications of a concentration-response relationship when analyses were stratified by cumulative blood Pb index, peak blood Pb, or other exposure indices.

In a study of 1,261 male newspaper linotype operators working in 1961 and followed until 1984, 38% had died from all causes (Michaels et al., 1991). Compared to the New York City population reference group, there was a marginally significant increased standardized mortality ratio in the printers of 1.35 (95% CI: 0.98, 1.82) for cerebrovascular disease (ICD-8 430-438), which became highly significant in those with 30 or more years exposure (1.68 [95% CI: 1.18, 2.31]; 37 of the total 43 deaths due to cerebrovascular disease). Atherosclerotic heart disease (ICD-8 410-414) mortality in printers was significantly below that expected from the general population, with a standardized mortality ratio of 0.63 (95% CI: 0.59, 0.73).

Mortality studies need to follow large groups over extended periods to achieve adequate statistical power. When large groups with well characterized exposure are followed for long periods, results of mortality studies assess the effects of long cumulative Pb exposure. Without detailed exposure histories stretching over decades, it is nearly impossible to determine if past

peak Pb exposure, time-integrated Pb exposure or average Pb exposure plays the critical role in producing greater then expected mortality. Noting that both population and occupational Pb exposures were at greater than current levels when the mortality studies reported here were begun, one can expect a 20 to 30 year lapse before one could assess any effects of current population and occupational Pb exposure on cardiovascular morbidity and mortality.

6.5.3.3 Other Cardiovascular Effects

Peripheral arterial disease (PAD), flow-limiting atherosclerosis in lower limb muscular arteries, was studied using Phase 1 (1999-2000) of the NHANES IV, the most recent NHANES dataset (Navas-Acien et al., 2004). PAD was categorized as a ratio of brachial artery (arm) systolic blood pressure to posterior tibial artery (ankle) systolic blood pressure < 0.90, with 139 subjects being classified as having PAD; there were 1,986 subjects without the disease. Blood Pb was classified by quartile, with the 1st quartile containing subjects with blood Pb <1.4 µg/dL and the 4th quartile containing subjects with blood Pb >2.9 µg/dL. Age ranged from 40 to >70 years. Three sets of covariates were tested in separate models. The first set, common to all models, included age, sex, race, and education. The second set included the first set and added BMI, alcohol intake, hypertension, diabetes, hypercholesterolemia, and glomerular filtration rate. The third set added self-reported smoking status and serum cotinine. Compared to first quartile blood Pb, 4th quartile blood Pb subjects had significant odds ratios for PAD of 3.78 (95% CI: 1.08, 13.19) and 4.07 (95% CI: 1.21, 13.73) for the first two models. The odds ratio of 2.88 (0.87, 9.47) for the third model was not statistically significant. However, the increasing odds ratio trend from 1st through 4th quartile was significant for all 3 models ($p < 0.02$).

The associations of umbilical cord blood Pb levels with pregnancy hypertension and blood pressure during labor were assessed in 3,851 women whose babies were delivered at the Boston Hospital for Women (Rabinowitz et al., 1987). The mean cord blood Pb level was 6.9 µg/dL (SD 3.3, range 0-35). Blood Pb concentrations were log transformed after adding one because some values were zero and the distribution was skewed. In a multivariate model adjusting for hematocrit levels, diabetes, ponderal index, race, tobacco use, and birth weight, cord blood Pb levels were significantly associated with systolic blood pressure during labor. A 10 µg/dL increase in cord blood Pb was associated with an increase of about 3 mm Hg in

systolic blood pressure. Significant associations were also observed between cord blood Pb levels and pregnancy hypertension. Compared to a cord blood Pb level of 0.7 µg/dL, the relative risk of pregnancy hypertension was 1.7 (95% CI: 1.3, 2.1) at a blood Pb level of 6.3 µg/dL and 2.2 (95% CI: 1.5, 2.9) at 15 µg/dL. Cord blood Pb levels were not found to be associated with preexisting hypertension or preeclampsia.

6.5.4 Lead and Cardiovascular Function in Children

Despite the potential importance of identifying the effects of Pb on cardiovascular function in children, only three studies addressed the issue. These studies are summarized in Annex Table AX6-5.4 and reviewed here. Factor-Litvak et al. (1996) studied the association of blood Pb on blood pressure in 260 children at age 5.5 years from a prospective study of Pb on child development from two cities in Serbia. They used multiple linear regression of contemporary linear blood Pb (range 4.1-76.4 µg/dL) on systolic and diastolic blood pressure, apparently stepwise as diastolic and systolic models had different covariates. Systolic models controlled for height, BMI, gender, ethnic group, and birth order, while diastolic models controlled for waist circumference, ethnic group, and birth order. Additional models further adjusting for maternal blood pressure, maternal hemoglobin, and for town were also presented, but not discussed here. Every 1 µg/dL increase in blood Pb was associated with 0.054 mm Hg and 0.042 mm Hg increase of systolic and diastolic blood pressure, respectively. Though no diagnostics were reported, the authors did try combined linear and quadratic blood Pb terms in alternative models and found the quadratic term to be nonsignificant. These marginally significant results may partially obscure early indications of altered cardiovascular health in young children exposed to Pb due to small sample size and use of linear Pb and stepwise regression in models.

Gump et al. (2005) studied blood Pb effects on both resting and induced-stress cardiovascular function in 122 children 9.5 years old from Oswego, NY. The effect of linear cord blood Pb (mean 3.0 µg/dL [SD 1.8]; range not given) and contemporary blood Pb (range 1.5-13.1 µg/dL) on blood pressure and other cardiovascular functions were tested in stepwise multiple regression models. The response to stress was evaluated by taking the difference between baseline and post-stress scores on the cardiovascular evaluations. Diastolic blood pressure change from baseline to stress condition increased 0.07 mm Hg for every 1 µg/dL

increase in contemporary blood Pb. Change in total peripheral resistance between baseline and stress conditions was 0.09 dyn-s/cm^3 for every 1 μg/dL increase in contemporary blood Pb. Authors reported testing blood Pb with linear, quadratic, and cubic terms that "did not add significantly to the prediction of these cardiovascular effects." Nonetheless, the scatterplot of the Pb effect on peripheral resistance change shows a notable nonlinear effect, as does an alternative analysis of the same outcome using blood Pb quartiles, in which a significant effect was seen between the group with 1.5 to 2.8 μg/dL and all higher blood Pb groups, as might be expected from a log-linear like blood Pb-peripheral resistance dose-response curve. The stepwise modeling technique and small sample size likely combined to over-fitting of the models. For instance, the total vascular resistance model had 12 covariates. The model might be difficult to replicate with an independent sample. Due to the study location, investigators had reason to believe that the children also had significant exposure to Hg and pesticide residues from eating contaminated lake fish.

A randomized succimer chelation trial of 780 12- to 33-month-old children with baseline blood Pb from 20 to 44 μg/dL resulted in significantly lower blood Pb in the treated group for only the first 9 to 10 months of the 60-month follow up period (Chen et al., 2006). No difference in blood pressure was noted between succimer and placebo groups during treatment. However, longitudinal mixed models showed systolic blood pressure to be 1.09 mm Hg (95% CI: 0.27, 1.90) higher in the succimer treated group than in the placebo treated group from month 12–60 of follow up and near zero coefficient for diastolic blood pressure. Cross-sectional regression analyses of blood pressure and linear blood Pb adjusted for clinical center, treatment group, race, sex, parents' education, single parent, age at test, height and BMI during follow up revealed near-zero Pb coefficients for systolic and diastolic blood pressure. The authors note the short duration of significant blood Pb difference between the groups, the overall downward trend in blood Pb with age in both groups, and the relatively short duration of elevated blood Pb in both groups as possible factors for the non-significant results. The lack of positive chelation effect observed in this study mirrors the results of chelation trials in the same children that showed no benefit in IQ and neurobehavioral performance. No diagnostics were mentioned, though non-parametric fitting of blood pressure and blood Pb with non-adjusted data were shown.

6.5.5 Potential Confounding of the Cardiovascular Effects of Lead

6.5.5.1 Confounding by Copollutants

High on the list of other metals that might be associated with cardiovascular disease is Cd, through its known effects on kidney function. If blood Pb and blood Cd strongly covary in a sample by sharing a common source (e.g., when the study sample is drawn from a population living near a nonferrous smelter emitting both metals), including simultaneous blood Pb and Cd measurements in the same model would likely show a significant reduction in both coefficients when compared to either metal alone. If, however, blood Cd and Pb do not covary in the sample, their coefficients in the model together would be similar to when tested separately. In a study of PAD (Navas-Acien et al., 2004) discussed in Section 6.5.3.3, investigators not only tested both Pb and Cd in separate models but also tested them simultaneously. In addition, they tested possible interactions between Pb and Cd, and between the two metals and sex, race-ethnicity, smoking status, renal function, and C-reactive protein. The correlation coefficient between natural log Pb and natural log Cd was 0.32 ($p < 0.001$), highly significant, though leaving 90% of the variance between them unexplained. When blood Pb and blood Cd were in the same model together, they both had significant trends of increasing odds ratios with increasing quartile of each metal. However, the nonsignificant point estimate of the odds ratio for blood Pb comparing the 1st and 4th quartile decreased when Cd was also included in the model (odds ratio of 2.88 versus 2.52). The odds ratio for Cd comparing the 1st and 4th quartile showed a similar decrease when modeled with Pb (odds ratio of 2.82 versus 2.42), but both point estimates remained significant. Thus, though point estimates of both Pb and Cd were approximately the same whether tested alone or together, the larger variance associated with the Pb coefficients rendered them nonsignificant. Part of the difference in variance between the two metals could be explained by noting that the reference group (lowest quartile) for Pb contained a little over half the number of subjects (n = 472; 18 cases, 454 noncases) than the reference group for Cd (n = 856; 27 cases, 829 noncases). The odds ratios for PAD with smoking status dropped from 4.13 (95% CI: 1.87, 9.12) to 3.38 (95% CI: 1.56, 7.35) when Pb was added to the model, but both odds ratios remained highly significant and the difference was not statistically tested. The failure to find a significant interaction between the two metals and between smoking status and both metals suggests that none of the odds ratio changes discussed above were significant. The same pattern of results was found when using cotinine blood levels instead of self-reported

smoking habit. Adding Cd alone or Cd and Pb together resulted in nonsignificant odds ratios for both indices of smoking.

The Belgian Cadmibel studies also were ideally situated to test possible interactions between blood Pb and Cd, but the technique of stepwise addition of variables to the multiple regression models of blood pressure did not allow retention of both metal variables together in the same model (Staessen et al., 1996b). From the lack of both Cd and Pb in any one model, it can be inferred that, if both variables had been forced into the model together, they both would have had nonsignificant coefficients.

6.5.5.2 Confounding by Smoking Status

Most studies reviewed in this section have controlled for tobacco use, where it often appears related to lower blood pressure. The majority of reviewed studies including smoking as a covariate never present the coefficients of smoking or related covariates. Only the Navas-Acien et al. (2004) study discussed in the previous section systematically addressed the issues related to possible confounding or effect modification with tobacco use.

6.5.5.3 Confounding by Alcohol Consumption

Possible confounding by alcohol use, generally associated with increased blood pressure, was discussed in the 1990 Supplement (Grandjean et al., 1989). Alcohol, especially in Europe, contained substantial Pb during much of the 20th century. This can be seen in the MONICA Augsberg, Germany cohort study (Hense et al., 1994). The study group was stratified by sex and then, only in men, by rural-urban location. Within each strata, the blood Pb range differed by alcohol use. In women, for example, the 10th and 90th percentile values of blood Pb (as estimated from graphs) were ~3.5 and 8.5 µg/dL for self-reported abstainers, 4.5 and 10.5 µg/dL in those drinking from 1 to 39 g/day, and 6.0 to 14.0 µg/dL in those drinking 40 plus g/day. Despite the finding that only women in the highest alcohol-use group had a significant Pb effect, it cannot be determined if the increase in Pb coefficient is significant, because the three coefficients associated with use of alcohol strata were not tested for differences among themselves; they were only tested for their significance from the null hypothesis of 0. Another study was based on subjects from the New Risk Factors Survey from the area around Rome, intended to determine confounding effects of a number of social and biochemical variables on

the blood Pb-blood pressure relationship (Menditto et al., 1994). Alcohol consumption, as well as BMI, heart rate, non-HDL cholesterol, and HDL cholesterol, triglycerides, cigarettes smoked/day, and skinfold thickness were all examined. A doubling of blood Pb was associated with an increase of 4.71 mm Hg in systolic and 1.25 mm Hg in diastolic blood pressure. As covariates were successively added to the model, the systolic coefficient was 4.6 (+BMI), 4.9 (+age), 5.1 (+heart rate), 4.3 (+high density lipids), 4.2 (+triglycerides), 3.9 (+glucose), 4.4 (+cigarettes/day), 4.1 (+skinfold), and 3.9 (+non-high density lipids). Similar changes were found upon adding covariates to the diastolic model. Alcohol never entered the models, but was significantly and positively associated blood Pb in bivariate testing. Unfortunately, neither standard errors or confidence intervals were given and the significance of the changes in the Pb coefficient could not be determined.

Alcohol as a true confounding variable is likely limited to studies in areas where alcohol contributes significantly to blood Pb. In a study of 249 bus drivers in San Francisco, CA, natural log Pb coefficients against blood pressure changed less than 10% when alcohol use was included as a covariate (Sharp et al., 1990). Blood Pb according to alcohol use was not reported. Still another study based on a U.S. population found a significant increase in blood Pb of a mixed group of males and females according to alcohol use, ranging from mean blood Pb of 7.3 µg/dL in nonusers to 9.2 µg/dL in those reporting more than 2 ounces/day over 3 days (Morris et al., 1990), with no report of significant effects of alcohol on blood pressure.

6.5.5.4 Confounding by Dietary Calcium Intake

The main thrust of the previously reported Morris et al. (1990) study was to examine the effects of dietary calcium on the effect of Pb on blood pressure in 78 males and 64 females, 18 to 80 years old, many of whom were hypertensive (undisclosed number), though those using medications for hypertension discontinued their use 1 month before testing started. Subjects were excluded if they had "secondary hypertension." The investigators measured serum calcium and assessed dietary calcium intake, among other variables. There were no changes in blood Pb or blood pressure noted as a result of dietary calcium supplementation.

Proctor et al. (1996), using the Normative Aging Study, examined possible modification of the effect of natural log blood Pb (blood Pb range 0.5-35 µg/dL) on blood pressure in 798 men, aged 45 to 93 years, by dietary calcium intake assessed by food questionnaire.

The study used multiple regression models with a fixed set of covariates, including age and age^2, BMI, adjusted dietary calcium, exercise, smoking, alcohol use, sitting heart rate, and hematocrit. Increased blood Pb was significantly associated with diastolic blood pressure and systolic blood pressure. Only systolic blood pressure significantly decreased with increased dietary calcium (0.004 mm Hg decrease for every 1 mg/day increase of dietary calcium). The authors formed dichotomized calcium intake (cut point at 800 mg/day) and blood Pb (cut point at 15 µg/dL) variables to test the interaction between blood Pb and calcium on blood pressure. They did not find a significant interaction; nor did they show the interaction coefficients.

A study of a subset of the Cadmibel Study with 827 males and 821 females, age 20 to 88 years, selected from areas known to represent a wide range of Cd exposure, specifically studied total serum calcium interactions with blood Pb on blood pressure (Staessen et al., 1993). Stepwise regression models, selecting from log blood Pb, age and age^2, BMI, pulse rate, log serum gamma-glutamyltranspeptidase, serum calcium, log serum creatinine, urinary potassium, smoking, alcohol intake, contraceptive pill use (females only), and a menopause indicator variable (females only), were stratified by sex for systolic and diastolic blood pressure. The stepwise procedure resulted in models each with a different mix of covariates. Increased serum calcium was significantly associated with increased systolic blood pressure in both males and females. Every increase of one log unit of blood Pb was associated with nonsignificant changes in blood pressure in women, but with a significant decrease in systolic blood pressure in men (systolic log blood Pb $\beta = -5.2$). A separate set of models were constructed with an interaction term between serum calcium and log blood Pb (details not shown). In women only, both main effects of Pb and calcium and the interaction effect were significant (no coefficients presented). At the 25th percentile of serum calcium (2.31 µmol/L), a doubling of blood Pb was associated with a 1.0 mm Hg increase in systolic blood pressure. At the 75th percentile of serum calcium (2.42 µmol/L) a doubling of blood Pb was associated with a 1.5 mm Hg increase in systolic blood pressure. Furthermore, serum calcium may itself be confounded with age in women, as women showed a sharp rise in serum calcium in their sixth decade of life, coincident with menopause, whereas the trend for serum calcium in men was steadily downward for each subsequent decade of age. The authors did not test an interaction term including calcium and age or calcium and menopausal status. Thus, the significant interaction effect between calcium and Pb on blood pressure may be a result of differences due to menopause.

6.5.5.5 Summary of Potential Confounding of the Lead Effect on Cardiovascular Health

The effects of Cd exposure, smoking, alcohol use, dietary and serum calcium levels have all been formally tested in a few studies, without significant effects as confounders of the Pb effect. Failure to find a significant confounding effect with Pb, however, does not argue to maintain these variables uncritically in models of blood pressure. If alcohol contains Pb, increased alcohol use will lead to increased blood Pb. In this case, both variables in the model will be collinear and this tends to distort estimated coefficients and standard errors of their effect on cardiovascular outcome. Tobacco use may influence Pb levels much more in occupational studies than in community exposure studies, especially if smoking in the factory is allowed. Frequent hand to mouth behavior will increase Pb exposure and, consequently, raise blood Pb concentrations. Serum calcium may statistically modify the Pb effect differentially by gender due to menopause in women. Menopause also affects Pb turnover. If serum calcium, blood Pb, and blood pressure are all statistically related, serum calcium should not be used in blood Pb-blood pressure/hypertension studies.

Epidemiologic studies cannot by themselves determine cause and effect relationships between Pb and cardiovascular disease. However, toxicological studies that observe similar phenomena in experimental animals give biological plausibility to the epidemiological results. In addition they may suggest mechanisms by which Pb might cause the observed epidemiologic effects. Chapter 5.5 details a series of results that run parallel to and give biological plausibility to the results in humans detailed in this section. In intact animals, elevated blood pressure develops only in response to continued exposure to Pb. If duration of Pb exposure is key to Pb-induced hypertension, as suggested by the consistently observed elevation of blood pressure and increased risk for hypertension associated with increased bone Pb (long term exposure) and the difficulty of detecting Pb effects on blood pressure in children, these animal studies argue that the Pb effects observed in humans are not the result of statistical artifact or confounding.

6.5.6 Gene-lead Interactions

Sodium-potassium adenosine triphosphatase α2 (ATP1A2) polymorphism was characterized in 220 workers formerly exposed to a mix of organic and inorganic Pb in the United States, noted above in other references (Glenn et al., 2001). The ATP1A2 (3′) one kilobase probe produced two homozygous (4.3/4.3 and 10.5/10.5) and one heterozygous

(4.3/10.5) genotypes and two homozygous (8.0/8.0 and 3.3/3.3) and one heterozygous (8.0/3.3) genotypes for the 2.5 kilobase ATP1A2 (5′) probe. Of the 209 subjects with data on both polymorphisms, 43.5% were doubly homozygous for 8.0/8.0 and 4.3/4.3, 34.4% were homozygous for 8.0/8.0 and heterozygous for 4.3/10.5, 11.5% were heterozygous for 8.0/3.3 and homozygous for 4.3/4.3, 5.3%. Also, 5.3% were doubly homozygous for 8.0/8.0-10.5/10.5, and 4.8% were doubly heterozygous for the two genotypes. Although only 13 African-American workers participated, prevalence of the 10.5 kilobase allele in the ATP1A2 (3′) genotype was statistically higher for them than for other races. Prevalence of hypertension (≥160/96 mm Hg or use of hypertension medication) was significantly higher in those with the 10.5/10.5 genotype than in others. When controlling for age, BMI, lifetime number of alcoholic drinks, the 10.5/10.5 genotype was associated with an odds ratio of 7.7 (95% CI: 1.9, 31.4) for hypertension as compared to the 4.3/4.3 homozygous genotype, but there were no effects of either blood Pb, tibia Pb, or their interaction with ATP1A2 (3′) genotype.

A multiple linear regression model for linear blood Pb and systolic blood pressure, controlling for age, use of hypertensive medication, current smoking, quartiles of lifetime alcohol consumption, and season, showed a significant main effect for 10.5/10.5 homozygous contrasted against combined 4.3/4.3 and 4.3/10.5 groups, associated with a 25.5 mm Hg reduction in blood pressure, primarily due to the limited blood Pb range for the homozygous group (maximum blood Pb of the 10.5/10.5 group 9 μg/dL; maximum blood Pb of the contrast group = 20 μg/dL). But the interaction between linear blood Pb and the 10.5/10.5 condition resulted in a significant increase of the blood Pb effect on blood pressure by 5.6 mm Hg for every 1 μg/dL blood Pb compared to the blood Pb effect in the other genotypes. The authors stated, but did not show analysis or coefficients, that the ATP1A3 (3′) polymorphism also significantly interacted with tibia Pb and systolic blood pressure. There were no significant relationships using the ATP1A2 (5′) gene. Thus, the ATP1A2 (3′) polymorphism appears to directly influence both prevalence of hypertension and the effect of Pb on blood pressure, though the small group (n = 9 with all measures) with the important 10.5/10.5 homozygous pattern would argue for enlarging this important study.

Another research group focused on polymorphisms of two genes suspected to be involved in Pb toxicokinetics, VDR and ALAD (Lee et al., 2001). Polymorphism of both genes is well studied and prevalence appears to be associated with race or ethnic background. Nearly

800 Korean workers aged 18 to 65 years (79.4% males) from Pb-using businesses were classified according to ALAD polymorphism (1-1 [homozygous] versus 1-2 [heterozygous]) and VDR polymorphism (bb [predominant homozygous] versus Bb plus BB [infrequent polymorphisms]). The homozygous ALAD 1-1 polymorphism was found in 90.1% of the group and the homozygous bb one was found in 88.8% of the group. When compared to a smaller group of non-Pb-exposed workers, blood Pb concentration (mean exposed 32.0 µg/dL [range 4-86] mean nonexposed 5.3 µg/dL [range 2-10] and tibia Pb concentration mean exposed 37.2 µg/g [range −7 to 338]; and mean nonexposed 5.8 µg/dL [range −11 to 27]) were much higher. The study used stepwise multiple regression models, selecting covariates remaining significant in the models from among a large set of potential control and confounding variables. Potential confounders were also allowed to remain in the models if "there were substantive changes in the coefficients of predictor variables" with their addition. Systolic models controlled for age and age^2, sex, BMI, antihypertensive medication use, and cumulative lifetime alcohol use. Depending on the presence or absence of linear blood Pb, tibia Pb, and DMSA-chelatable Pb in the models, and the gene-age interactions tested, blood urea was added to the model. Diastolic models controlled for age, sex, BMI, cumulative alcohol consumption, and linear blood Pb. Hypertension (systolic >160 mm Hg or diastolic >96 mm Hg) logistic multiple regression models controlled for age, sex, BMI, tibia Pb, and current alcohol use. Among the exposed workers bb VDR genotypes had significantly lower DMSA-chelatable blood Pb and lower diastolic and systolic blood pressure than the combined Bb and BB genotypes. The only significant interaction reported between predictor variables and gene polymorphism on blood pressure was with the VDR polymorphism bb allele, which had a less pronounced increase in systolic blood pressure with age than subjects with the B allele. There were only marginally significant associations of systolic blood pressure with tibia Pb and linear blood Pb. There were no significant associations in models of diastolic blood pressure with linear blood Pb, DMSA-chelatable blood Pb, or tibia Pb. Tibia Pb was significantly associated with hypertension (odds ratio of 1.05 [95% CI: 1.00, 1.12] for each 10 µg/dL increase in tibia Pb). Workers with VDR B allele had significantly higher prevalence of hypertension (odds ratio = 2.1 [95% CI: 1.0, 4.4]) than workers with the bb genotype, but no other Pb variable or interaction with VDR status was reported significant. Though VDR status was significantly related to blood pressure and

prevalence of hypertension, there were no significant effects of ALAD polymorphism on blood pressure or hypertension or of VDR interactions with any Pb exposure variable.

Lustberg et al. (2004) studied the same Korean Pb workers (n = 793) to examine relationships between the G^{894}–T^{894} polymorphism in the gene regulating endothelial nitric oxide synthase (eNOS) and blood Pb effects on blood pressure and hypertension. Nitric oxide metabolism has been suggested both as a mechanism for altered blood pressure and for moderating the effects of Pb on blood pressure, though there is experimental support for and against both hypotheses. After classifying subjects as homogenous for the GG type (85%), heterogeneous for both types (TG) (14%), or homogenous for TT (1%), the TG and TT types were combined into a single group (TG/TT). Diastolic and systolic multiple regression models were constructed with a fixed set of covariates, including smoking, alcohol consumption, age, sex, BMI, and education. Logistic regression models used blood pressure criteria of either ≥140 mm Hg diastolic blood pressure, ≥90 mm Hg systolic blood pressure, or self-report of using antihypertensive medications. There was no effect of genotype on diastolic or systolic blood pressure or on hypertension prevalence in multiple regression models, nor any significant interaction of Pb exposure indices with gene status.

Because interaction testing in statistical models optimally requires balanced groups for uncomplicated interpretation, further gene-Pb interaction exploration should use studies with nearly equal numbers of heterogeneous and homogenous groups. Also, because adequate power for testing significant interactions requires large groups, subsequent studies should draw subjects from the general population. In addition to enlarging the potential subject pool, population studies may more easily avoid the selection biases often found in occupational studies.

6.5.7 Summary of the Epidemiologic Evidence for the Cardiovascular Effects of Lead

The combined blood Pb studies using blood pressure/hypertension as an outcome continue to support the conclusions of the 1990 Supplement that there is a positive association between blood Pb and increased blood pressure. The occasional finding of significant negative associations of blood Pb with blood pressure (e.g., the Cadmibel study, one NHANES III study, the postpartum phase of the Los Angeles pregnancy study) have not been adequately explained and require further confirmation and study

The most promising developments in this field since the 1990 Supplement have been the use of bone Pb as a long-term cumulative Pb exposure index and the introduction of genetic analysis into the studies as potential Pb effect modifiers. With one exception, all studies using bone Pb have found a consistently positive and significant effect on blood pressure and/or hypertension. The ability to estimate past exposure in cross-sectional studies is a significant advance. The results of the bone Pb studies to date highlight the important role of accumulated Pb exposure in the development of cardiovascular problems.

Animal toxicologic studies have found that cell, tissue, and organ response to Pb is immediate and may provide clues to the mechanisms by which Pb contributes to cardiovascular disease in humans. Lead interference in calcium-dependent processes, including ionic transport systems and signaling pathways important in vascular reactivity may only represent the first step in the cascade of Pb-induced physiological events that culminates in cardiovascular disease. Lead alteration of endothelial cell response to vascular damage, inducement of smooth muscle cell hyperplasia, alteration of hormonal and transmitter systems regulating vascular reactivity, and its clear role as promoter of oxidative stress suggest mechanisms that could explain the Pb-associated increase in blood pressure, hypertension, and cardiovascular disease noted in this section.

- Studies support the relationship between increased Pb exposure and increased adverse cardiovascular outcome, including increased blood pressure, increased incidence of hypertension, and cardiovascular morbidity and mortality. For blood Pb and blood pressure, every doubling of blood Pb is associated with a ~1.0 mm Hg increased systolic and ~0.6 mm Hg increased diastolic blood pressure for blood Pb between 1 and >40 µg/dL.

- Cumulative past Pb exposure, measured by bone Pb, may be more important than present exposure in assessing cardiovascular effects of Pb exposure. Over the range of bone Pb concentration of <1.0 µg/g to 96 µg/g, every 10 µg/g increase in bone Pb was associated with increased odds ratio of hypertension between 1.28 and 1.86, depending upon the study. Two studies measured averaged increased systolic blood pressure of ~0.75 mm Hg for every 10 µg/g increase in bone Pb concentration over a range of <1 to 52 µg/g.

- Although females often show lower Pb coefficients than males, and Blacks higher Pb coefficients than Whites, where these differences have been formally tested, they are usually not statistically significant. The tendencies may well arise in the differential Pb exposure in these strata, lower in women than in men, higher in Blacks than in Whites. The same sex and race differential is found with blood pressure.

- Though genotyping has not yet produced results predicting differential cardiovascular response to Pb, this field has potential to identify individuals at higher risk of adverse Pb effects.

6.6 REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF LEAD

6.6.1 Summary of Key Findings of the Reproductive and Developmental Effects of Lead from the 1986 Lead AQCD

Lead has been implicated as a risk factor for reproductive outcomes for over a century Rom, 1976; Oliver, 1911). As early as 1860, increased rates of stillbirths and spontaneous abortions were found in women with occupational Pb exposure (usually in the ceramics industry) and in women with husbands employed in the Pb industry, compared to unexposed women (Rom, 1976). Other early investigations found increased rates of physically and mentally "retarded" offspring among these same groups. In 1910, these findings resulted in the first Pb-related occupational regulation; the British Committee on Occupational Health recommended that women not be employed in the Pb industry (Oliver, 1911). These observations, however, were based on exposure levels far above those considered acceptable today, and current research now focuses on substantially lower exposure levels.

The 1986 Lead AQCD provided evidence that Pb, at high exposure levels, exerted significant adverse health effects on male reproductive functions. Several studies observed aberrations in both sperm count and morphology in men occupationally exposed to relatively high levels of Pb (blood Pb levels of 40-50 µg/dL). However, the effects of Pb on female reproductive function and fetal growth were suggestive but equivocal, perhaps due to the small sample sizes and inadequate controlling for potential confounding factors.

This section provides a critical review of the literature regarding the associations between exposure to environmental Pb and reproductive outcomes. First, the evidence for the placental transfer of Pb is reviewed; this is key to providing a basis and mechanism for fetal exposure. Second, associations between Pb exposure and various outcomes are reviewed. Outcomes of interest are reproductive function (fertility), spontaneous abortion, fetal growth, preterm delivery, and congenital anomalies. Each section below begins with a summary of the literature up to

1986, the year of the last Lead AQCD. Then, key studies are reviewed and each section ends with a conclusion based on the evidence provided.

6.6.2 Placental Transfer of Lead

In 1969, Barltrop (1969) demonstrated that Pb crosses the placenta beginning as early as gestational week 12 and that the transfer rate then increased to term. Pb accumulations were found in the bones, livers, blood, hearts, kidneys, and brains of stillborn and spontaneously aborted fetuses. These observations were replicated by numerous investigators. For example, Casey and Robinson (1978) found Pb accumulations in the livers, kidneys, and brains of stillborn fetuses. Lead accumulations were also found in the livers, brains and kidneys of first trimester-aborted fetuses (Chaube et al., 1972), suggesting placental transfer of Pb earlier than 12 weeks of gestation. Newer findings, published since 1986, are summarized in Annex Table AX6-6.1 and are assessed below.

Placental transfer of Pb is confirmed by correlations of maternal blood Pb, umbilical cord blood Pb, and placental Pb concentrations in a variety of settings. Umbilical cord blood reflects fetal blood. Early studies, prior to 1986, found correlation coefficients between maternal and umbilical cord blood Pb ranging from 0.5 to 0.8 (all highly statistically significant). More recent studies also found significant correlations between maternal and fetal blood Pb. For example, a prospective study in Kosovo, Yugoslavia recruited 1,502 women at mid-pregnancy in two towns—one with high exposure due to the presence of a Pb smelter, refinery, and battery plant, and one with relatively low Pb exposure. The correlation between maternal blood Pb (either at delivery or at mid-pregnancy) and cord blood Pb ranged from 0.8 to 0.9 (Graziano et al., 1990). Among women with substantially lower levels of exposure (e.g., blood Pb 1.9 μg/dL) the correlation between maternal and cord blood Pb was 0.79 (Harville et al., 2005).

Chuang et al. (2001) propose that while maternal and cord whole blood Pb are highly related, fetal exposure may be even more influenced by maternal plasma Pb. Using data from a cohort of 615 women in Mexico City recruited in 1994–1995, these investigators used structural equation modeling to estimate the associations of cord blood Pb with whole blood Pb, bone Pb (cortical and trabecular), and the latent variable, plasma Pb. They found that maternal plasma Pb had a stronger association with cord blood Pb compared to maternal whole blood Pb.
The greatest contributors to plasma Pb were bone Pb and airborne Pb. However, with declining

exogenous Pb exposure, these investigators note that the measurement of plasma and bone Pb may become increasingly important in assessing fetal exposure.

These data provide little doubt of fetal exposure to Pb via placental transport. Further, it appears that Pb crosses the placenta throughout pregnancy, leading to continual exposure of the fetus. Indeed, there is evidence to suggest that maternal blood Pb levels during the later half of gestation increase (Gulson et al., 2004; Hertz-Picciotto et al., 2000; Rothenberg et al., 1994; Sowers et al., 2002). The magnitude of the increase ranges from 14 to 40%, possibly due to the different starting blood Pb in each study (Bellinger, 2005). The increase in blood Pb in the later half of pregnancy may result from physiologic changes in maternal homeostasis during pregnancy and, in particular, to mobilization of Pb stores from other body organs (Bellinger, 2005). Indirect evidence for such mobilization comes from the increased rate of bone turnover during the later half of gestation, prompted by the increased fetal need for calcium (Moline et al., 2000). Thus, both the epidemiological evidence and the biological plausibility of the associations support the role of maternal-fetal transfer of Pb.

Additionally, in populations with greater Pb burdens, the fetus may be at even greater increased risk for exposure and possible adverse effects of exposure. Among the variables associated with Pb exposure in pregnant (and nonpregnant) women are: smoking and alcohol consumption (Graziano et al., 1990; Rhainds and Levallois, 1997), pica (Rothenberg et al., 1999), use of ethnic remedies and cosmetics (Al-Ashban et al., 2004; CDC, 1993), and food preparation in inappropriately Pb-glazed pottery (Azcona-Cruz et al., 2000; Rothenberg et al., 2000). There is some evidence that low calcium intake is also associated with higher blood Pb (Gulson et al., 2004; Hernandez-Avila et al., 2003; Hertz-Picciotto et al., 2000). Finally, the location where the mother resides (or resided as a child) may increase blood Pb (Graziano et al., 1990). Blood Pb levels are elevated among U.S. immigrants, especially those who migrated from countries where Pb is still used as a gasoline additive (CDC, 2000); indeed, blood Pb levels are inversely associated with the number of years since migration (CDC, 2000; Klitzman et al., 2002; Rothenberg et al., 1999).

In conclusion, the epidemiologic evidence indicates that Pb freely crosses the placenta, resulting in continued fetal exposure throughout pregnancy. Indeed, the evidence is strong that exposure increases during the later half of pregnancy. Exposure to the fetus is more pronounced

in high-risk populations, especially those who migrated from countries still using Pb as a gasoline additive.

6.6.3 Effects of Lead on Reproductive Function

6.6.3.1 Effects on Male Reproductive Function

Male reproductive function is measured using the reproductive history of the male (i.e., number of pregnancies fathered), time to pregnancy and direct measures of semen quality (usually sperm count, motility and morphology). Most studies relating Pb exposure to male reproductive function are based on data collected in the occupational setting linked to population birth registries and on studies directly collecting questionnaire exposure and outcome data.

6.6.3.1.1 Sperm Count, Motility and Morphology

Recent publications which purport a decline in sperm concentration, motility, and morphology seek the explanation in the rising use of man-made chemical endocrine disruptors (Auger et al., 1995; Fisch et al., 1997; Farrow, 1994; Gyllenborg et al., 1999; Kavlock et al., 1996; Keiding et al., 1994; Keiding and Skakkebaek, 1996; Lerchl, 1995; Olsen et al., 1995; Sherins, 1995). Several studies from the 1970s and early 1980s suggest aberrations in both sperm count and morphology in men exposed to relatively high levels of Pb. Results from these studies as well as more recent studies are summarized in Annex Table AX6-6.2. In the earliest study, Lancranjan et al. (1975) found decreased sperm counts and an increased prevalence of morphologically abnormal sperm among workers heavily exposed to Pb (mean blood Pb 74.5 µg/dL) as well as those moderately exposed (mean blood Pb 52.8 µg/dL). These findings have been corroborated by results of studies in the United States (Cullen et al., 1984) and Italy (Assennato et al., 1986) which describe similar effects in workers with blood Pb levels above 60 µg/dL.

More recently, corroborating data was described in a comprehensive review by Apostoli et al. (1998). In studies of men with blood Pb levels above 40 µg/dL, decreases in sperm count and concentration, motility and morphologic aberrations were found. Chowdhury et al. (1986) found a significant decrease in sperm count and motility and an increase in the number of sperm with abnormal morphology in 10 men with occupational Pb exposure; the average blood Pb in the exposed group was 42.5 µg/dL compared to 14.8 µg/dL in the unexposed. Similar results

were found in a group of 30 Pb-exposed factory workers compared to controls (Lerda, 1992). In a large study of male Pb smelter workers, Alexander et al. (1996a) found a decreasing trend of sperm concentrations with increasing Pb exposure. In this cohort, 152 workers provided blood specimens and 119 also provided semen samples. Geometric mean sperm concentrations were 79.1, 56.5, 62.7, and 44.4 million cells/mL for blood Pb levels of <15, 15-24, 25-39, and ≥40 μg/dL, respectively. Long-term body Pb burden was estimated from current blood Pb concentrations and historical blood Pb monitoring data. Using this measure of long-term Pb body burden, a similar trend was found for sperm concentration, total sperm count, and total motile sperm count. No associations were found for sperm morphology or serum concentrations of reproductive hormones. A study of traffic police in Peru, where leaded gasoline is still in use, found decreases in sperm morphology, concentration, motility and viability among men with blood Pb ≥40 μg/dL compared to men with blood Pb <40 μg/dL.

Using data from an international study of 503 men employed in the Pb industry, Bonde et al. (2002) considered the lowest adverse effect level associated with perturbed semen parameters. Median sperm concentration was reduced by 49% in men with blood Pb >50 μg/dL; regression analysis indicated a threshold value of 44 μg/dL. These investigators conclude that adverse effects on sperm quality were unlikely at blood Pb levels <45 μg/dL.

In a population of couples undergoing either artificial insemination or in vitro fertilization, Benoff et al. (2003a,b) found higher concentrations of Pb in seminal fluid in the male partner among couples who did not conceive, compared to those who did conceive. While not directly measuring the adverse effects of Pb on sperm per se, these data suggest a possible mechanism for the transfer of Pb from paternal exposure to the fetal environment. Hernández-Ochoa et al. (2005) also provide evidence that Pb concentrations in seminal fluid may be a better indicator of exposure than blood Pb. Mean blood Pb in this sample was lower than in most other studies, 9.3 μg/dL. Decreases in sperm concentration, motility, morphology, and viability were correlated with seminal fluid Pb or Pb in spermatozoa, but not with blood Pb.

Overall, the available evidence suggests a small association between exposure to Pb, usually in the workplace, and perturbed semen quality. It appears that sperm count and morphology (% normal forms) may be decreased at exposures >45 μg/dL. Future research should focus on studies of men exposed to lower levels of Pb, as exposures in the very high range are associated primarily with occupational exposure. These studies should also account for

variables known to be associated with semen quality and which may also be associated with exposure, e.g., social class, other environmental exposures such as heat and vibration, and lifestyle variables such as cigarette smoking and alcohol use.

6.6.3.1.2 Time to Pregnancy

Time to pregnancy represents a sensitive measure of fecundity. Time to pregnancy is important because it measures the end effect of perturbed reproductive function. While it is important and necessary to understand the associations between prenatal exposures and endocrine abnormalities and semen characteristics, they represent possible antecedents to the occurrence of pregnancy. Previous reports demonstrate good validity and reliability for reports of time to pregnancy in both males and females and when time to recall has been both long and short (Weinberg et al., 1993, 1994).

One advantage to the use of this parameter, as compared to just an infertility measure, is that it does not require categorization of men into fertile and infertile groups. Among couples that succeed in establishing pregnancy, there is considerable variability in the time between discontinuation of contraception and conception (Weinberg et al., 1994). With the possible exception of cigarette smoking and age, little is known regarding such intercouple variability. Delays in time to pregnancy may be indicative of a range of reproductive abnormalities of both partners, including impaired gametogenesis, hormonal disruptions, and very early unrecognized pregnancy loss. Time to pregnancy has the menstrual cycle as its natural unit and is thus measured in integer units of menstrual cycles.

Usually, time to the most recent pregnancy is taken as the outcome (Baird et al., 1986). The measure of exposure in these studies usually is the fecundity density ratio, which is similar to an incidence density ratio. Fecundity density ratios can be interpreted as the risk of pregnancy among the exposed during an interval, compared to the risk of pregnancy among the unexposed during the same interval. In such studies, the intervals of interest are menstrual cycles. Fecundity density ratios less than one indicate reduced fecundity (i.e., longer time to pregnancy) among the exposed compared to the unexposed, while those greater than one indicate enhanced fecundity (i.e., shorter time to pregnancy) in the exposed. Usually fecundity density ratios are calculated using discrete time Cox proportional hazards regression models.

Several recent studies evaluate time to pregnancy when the male partner is occupationally exposed to Pb. These studies are summarized in Annex Table AX6-6.3 and reviewed here. The Asclepios Project, a large European collaborative cross-sectional study, evaluated time to pregnancy in 1,108 men of whom 638 were exposed to Pb (Joffe et al., 2003). The reference group consisted of Pb workers for whom exposure did not coincide with time of pregnancy. The investigators only included pregnancies which resulted in live births. Fecundity density ratios were 1.12 (95% CI: 0.84, 1.49), 0.96 (95% CI: 0.77, 1.19), 0.88 (95% CI: 0.70, 1.10) and 0.93 (95% CI: 0.76, 1.15) for blood Pb levels <20, 20-29, 30-39, and ≥40 μg/dL, respectively. These results indicate that no association was found between blood Pb and delayed time to pregnancy. Similar results were found when duration of exposure or cumulative exposure was used as the exposure metric.

A separate report was published in the Italian group of men included in the Asclepios project (Apostoli et al., 2000). Blood Pb at the time closest to conception was used as the measure of exposure. Lead-exposed men (n = 251) who had experienced at least one completed pregnancy were compared to nonexposed men (n = 45). Contrary to what was expected, time to pregnancy was significantly shorter among couples in which the male partner was exposed to Pb compared to those in which the male partner was not exposed. In secondary analyses, time to pregnancy was longer among men with the highest blood Pb (i.e., ≥40 μg/dL). Limiting the analysis solely to exposed men, time to pregnancy was longer among men with higher blood Pb levels.

Among 502 couples identified by Sallmén et al. (2000) from the Finnish Institute of Occupational Health in which the male partner was exposed to Pb, time to pregnancy was reduced among those with blood Pb >10 μg/dL compared to those with blood Pb ≤10 μg/dL. However, when blood Pb was stratified, no concentration-response relationship was found. Fecundity density ratios were 0.92 (95% CI: 0.73, 1.16), 0.89 (95% CI: 0.66, 1.20), 0.58 (95% CI: 0.33, 0.96) and 0.83 (95% CI: 0.50, 1.32) for exposures of 10-20, 21-30, 31-40, and ≥40 μg/dL, respectively. In this study, blood Pb concentrations close to the time of conception were available on 62% of men, while in 38% it was estimated using blood Pb levels obtained at other points or based on job descriptions.

Among 280 pregnancies in 133 couples in which the male partner was employed in a battery plant, 127 were conceived during exposure while the remainder conceived prior to

exposure (Shiau et al., 2004). Time to pregnancy increased with increasing blood Pb, especially when blood Pb levels were ≥30 μg/dL. Fecundity density ratios were 0.50 (95% CI: 0.34, 0.74) and 0.38 (95% CI: 0.26, 0.56) for blood Pb levels of 30-39 and >39 μg/dL, respectively. In 41 couples, one pregnancy occurred prior to exposure and one during exposure—time to pregnancy during exposure was significantly longer. Of note, this is the only study to estimate decreases in time to pregnancy when blood Pb was below 40 μg/dL; time to pregnancy increased by 0.15 months for each 1 μg/dL increase in blood Pb between 10 and 40 μg/dL.

6.6.3.1.3 Reproductive History

Since the 1986 Lead AQCD, several studies examining the association of Pb with reproductive history have been published. Results from these studies are presented in Annex Table AX6-6.4. Population-based birth registries in the Scandinavian countries provide data on medically diagnosed pregnancies. These registries provide a basis for linking occupational data on Pb exposure obtained by place and duration of employment or by direct measures of blood Pb relative to the timing of marriage or conception. Using a roster of men employed in three battery plants in Denmark, Bonde and Kolstad (1997) matched all births to the 1,349 employees when they were age 20-49 years. A control group of 9,656 men who were not employed in a Pb industry was chosen. No associations were found between employment or, among those employed in the Pb industry, duration of employment in the Pb industry and birth rate.

A similar study in Finland (Sallmén et al., 2000) examined the association between conception and blood Pb among men monitored for occupational exposure at the Finnish Institute of Occupational Health (n = 2,111). Men were categorized as probably exposed and possibly exposed based on their measured blood Pb in relation to the time of marriage. A nonexposed group of 681 men with blood Pb ≤10 μg/dL was similarly evaluated. Among men in the probable exposure group, the risk of failing to achieve a pregnancy increased with increased blood Pb in a monotonic concentration-response fashion. Compared to the nonexposed, the risk ranged from 1.3 to 1.9 for blood Pb levels 10-20 μg/dL and >50 μg/dL, respectively.

Lin et al. (1996) linked records from the Heavy Metal Registry in New York State to birth certificates from the New York State Office of Vital Statistics for the period 1981 to 1992. Exposure was defined as having at least one blood Pb measurement above 25 μg/dL and

identified 4,256 men. A reference group of 5,148 men was frequency matched for age and residence. The exposed group had fewer births than expected, and was especially pronounced among men employed in the Pb industry for over 5 years.

Among 365 men occupationally exposed to metals, Gennart et al. (1992) identified 74 exposed continuously for more than 1 year and with at least one blood Pb measurement >20 µg/dL. Compared to a reference group with no occupational exposure, the probability of at least one live birth was significantly reduced. Fertility decreased with increasing duration of exposure but no concentration-response relationship with blood Pb was found (possibly due to the small sample size of exposed men).

A study of men exposed to Pb in a French battery plant (Coste et al., 1991) reported no effect on fertility. However, this study did not adequately control for potentially confounding variables, particularly those related to the women. Further, nonexposed workers were defined as those with no recorded blood Pb values, which likely resulted in exposure misclassification.

One potential mechanism to explain the associations between Pb exposure and male reproductive outcomes may be through an effect of Pb on circulating pituitary and testicular hormones. Several studies have evaluated this hypothesis in groups of workers (Braunstein et al., 1978; Cullen et al., 1984; Erfurth et al., 2001; Ng et al., 1991; Rodamilans et al., 1988). Further discussions on the effect of Pb on male reproductive hormones are presented in Section 6.9.3.3.1. In general these studies find perturbations in concentrations of follicle stimulating hormone, luteinizing hormone, and testosterone. Although many of these studies were limited by small sample sizes, lack of control groups, and mixtures of exposures, taken together, they provide evidence for this possible mechanism.

6.6.3.1.4 Genotoxicity and Chromosomal Aberrations

The potential genotoxicity and ability to induce chromosomal aberrations speak to the mechanisms by which Pb is a potential reproductive toxin. Two possible mechanisms by which Pb may affect reproduction are through affinity with proteins and ability to mimic the actions of calcium (Silbergeld et al., 2000).

Data from occupational studies regarding the effects of Pb on chromosomes are contradictory; however, the bulk of evidence suggests that there may indeed be a genotoxic effect. Early studies in occupational groups find associations between Pb exposure and increased

frequency of sister chromatid exchanges (Grandjean et al., 1983; Huang et al., 1988; Leal-Garza et al., 1986; Mäki-Paakkanen et al., 1981). Similar results were found in a group of environmentally-exposed children with blood Pb levels ranging from 30 to 63 µg/dL (Dalpra et al., 1983). Increased frequencies of chromosomal aberrations, particularly chromatid aberrations, were found in battery plant workers and were correlated with increased blood Pb (Huang et al., 1988). A more marked increase was found when blood Pb were above 50 µg/dL. Other occupational studies find similar associations (Al-Hakkak et al., 1986; Forni et al., 1976, 1980; Nordenson et al., 1978; Schwanitz et al., 1970). Other studies find no evidence of chromosomal aberrations when blood Pb ranged from 38 to 120 µg/dL (Bauchinger et al., 1977; Mäki-Paakkanen et al., 1981; O'Riordan and Evans, 1974; Schmid et al., 1972; Schwanitz et al., 1975). More recently, two studies in battery plant workers (mean blood Pb 40.1 µg/dL) and controls (mean blood Pb 9.8 µg/dL) found an increase in high-frequency cells and sister chromatid exchanges among the workers, indicating the cytogenetic toxicity of Pb (Duydu et al., 2001, 2005). An increase in sister chromatid exchanges, although not statistically significant, was also found in individuals exposed to Pb and/or alcohol and tobacco (Rajah and Ahuja, 1995, 1996). In the Lithuanian populations exposed to either environmental or occupational Pb, a higher incidence of sister chromatid exchanges and chromosomal aberrations was found (Lazutka et al., 1999), although these populations were also exposed to other potentially genotoxic substances. Recent data also indicates that Pb may inhibit DNA repair responses among Pb-exposed workers (Karakaya et al., 2005).

Occupational exposure to Pb, particularly when blood Pb was high (i.e., >40 µg/dL), was associated with increased mitotic activity in peripheral lymphocytes and with an increased rate of abnormal mitosis (Forni et al., 1976; Minozzo et al., 2004; Sarto et al., 1978; Schwanitz et al., 1970). Again, to the extent these changes influence the production of gametes, this is a potential mechanism explaining associations between Pb exposure and decreased male fecundity.

6.6.3.1.5 Issues Concerning Studies of Male Fecundity Related to Lead Exposure

In examining studies of fecundity and fertility, several issues relating to interpretation and bias must be addressed. Infertility usually is defined as 12 months of continuous unprotected intercourse without pregnancy. Fecundity represents both a characteristic of the individuals and a characteristic of a couple, meaning that both partners must be biologically able to procreate.

Thus, one possible explanation for observations of reduced fecundity related to occupational Pb exposure in the male partner is the exposure he "takes home" via transport of dust on clothing and shoes, ultimately resulting in an effect related to the female partner. Other possible interpretations need to account for measurement error, especially related to the outcomes of reproductive history and time to pregnancy, bias in the selection of subjects for study, and the control for potentially confounding variables.

Both reproductive history and time to pregnancy are subject to errors of recall and rely on the veracity of the subject. Several studies have evaluated recall and veracity of the male partner using the female partner as the "gold standard." In general, these find good reliability between the male and female (Weinberg et al., 1993, 1994). Nevertheless, it is possible, at least for studies using men as the sole informant, that the number of pregnancies a man has fathered is underreported. If reporting is nondifferential with regard to Pb exposure, then associations will generally be biased towards the null value; however, since characteristics such as social circumstances, ethnicity, and age may affect both exposure and reporting, it is difficult to evaluate the role of bias.

It was not clear from many of the studies that men with medical conditions which affect fecundity/fertility were excluded. Further, several prescription and over-the-counter medications also affect fecundity as does a history of surgery in the genital area (e.g., varicocele). To the extent that these conditions are related to the absence of employment in Pb-industries, then the results may be subject to a type of "healthy worker" effect. Because it is unclear whether many of these studies asked about these conditions, this cannot be ruled out as a possible source of bias.

In retrospective studies it is often useful to use the outcome of the most recent pregnancy in the primary analysis. The reason for this is to reduce any possible recall bias. This type of bias may also be an issue in studies which use occupational registry data, i.e., men may have fathered an additional pregnancy after employment in the industry ceased.

Variables considered potential confounders in studies of fertility and fecundity include sociodemographic characteristics (e.g., age, ethnicity, education, occupation); prenatal and recent lifestyle variables such as cigarette smoking, alcohol use, and medication use; exposures through occupation and hobby, and recent medication use. Also important in these studies is control for

factors which may affect the partner's fertility, e.g., cigarette smoking. Many of the studies reviewed did not carefully measure or adjust for confounding variables.

The issues presented above potentially limit the interpretation of results from studies examining the association of Pb exposure with male fecundity and fertility. Nevertheless, most studies find small associations between Pb exposure at high levels (i.e., ≥45 μg/dL) and slightly reduced male fecundity or fertility.

6.6.3.2 Effects on Female Reproductive Function

Few data directly address the effects of Pb exposure on fecundity in the female. A recent retrospective study of time to pregnancy among wives of Pb workers provides limited support that Pb exposure is associated with increased time to pregnancy. Fecundity density ratios were 0.92 (95% CI: 0.72, 0.16), 0.89 (95% CI: 0.66, 1.20), 0.58 (95% CI: 0.33, 0.96), and 0.83 (95% CI: 0.50, 1.32) for blood Pb in the male partners of 10-20, 21-30, 31-38 and ≥39 μg/dL compared to <10 μg/dL, respectively. Note however, that exposure here is measured in the male partners and not the females.

Time to pregnancy was evaluated in 121 women biologically monitored for Pb exposure at the Finnish Institute of Occupational Health between 1973 and 1983 (Sallmén et al., 1995). Fecundity did not differ with level of exposure (defined as <10 μg/dL, 10-19 μg/dL and ≥20 μg/dL), but among women with blood Pb between 29 and 50 μg/dL, there was a suggestion of reduced fecundity (longer time to pregnancy). However, only a small number of subjects (n = 8) were exposed in this range.

In the limited number of studies, there is little evidence regarding the associations between Pb exposure and fertility in the female to draw any conclusions at this time.

6.6.4 Spontaneous Abortion

6.6.4.1 Spontaneous Abortion and Maternal Exposure to Lead

Historical observations suggest increased rates of spontaneous abortion among Pb-exposed women, particularly those employed in cottage industries (Rom, 1976). Two early studies in a smelter town in Sweden (Nordström et al., 1978a, 1979) suggest elevated rates of spontaneous abortion among female employees at the smelter and among female residents living in close proximity to the smelter. Neither of these studies used biological markers of

Pb exposure. Moreover, the Swedish smelter study included other exposures such as arsenic, zinc, and Cd; thus the conclusions for these analyses should be tempered.

In contrast, a prospective study in and around a smelter town in Port Pirie, Australia (McMichael et al., 1986) did not find an association between blood Pb concentration and spontaneous abortion. However, it was likely that complete ascertainment of spontaneous abortions was not obtained (Rowland and Wilcox, 1987), since most women were recruited for this study after the first trimester of pregnancy. A retrospective cohort study in two towns in the former Yugoslavia (Murphy et al., 1990) showed no associations between Pb exposure and spontaneous abortion in the first reported pregnancy. One of these towns was a smelter town with relatively high Pb exposure (at recruitment during mid-pregnancy, the mean blood Pb concentration was 17.1 μg/dL, while in the control town the mean blood Pb was 5.1 μg/dL). A similar study in Poland (Laudanski et al., 1991) evaluated the association between Pb-exposed and nonexposed areas for their reproductive histories. Among women in the exposed areas, 11% reported having at least one prior spontaneous abortion, compared to 19.5% of women in the unexposed areas.

Two studies in Finland (Lindbohm et al., 1991a; Taskinen, 1988) used hospital registry data to ascertain women with either spontaneous abortions or livebirths. Either maternal job histories (Taskinen, 1988) or both maternal and paternal job histories were obtained from a registry of occupational blood Pb measurements. Neither study found evidence of an association between maternal Pb exposure and spontaneous abortion. In the Lindbohm et al. (1991a) study, maternal exposure was extrapolated from the occupation of the father.

In Bulgaria, pregnant women residing in or near Pb smelting areas or petrochemical plants were prospectively followed for pregnancy outcomes (Tabacova and Balabaeva, 1993). The investigators compared blood Pb in those women with spontaneous abortions and those without. Blood Pb concentrations in cases were significantly higher than in controls (mean blood Pb 7.1 μg/dL versus 5.2 μg/dL, respectively). However, this study did not fully describe the selection of women nor the definition for cases.

Women employed by the U.S. Forest Service and exposed to Pb-based paint (to mark trees for clearing) were studied using self-reported questionnaires (Driscoll, 1998). Adjustment was made for potential confounders and generalized estimating equations were used to adjust for multiple pregnancies per woman. Significant associations were found for three types of paint

containing Pb pigment (odds ratios of 4.3 [95% CI: 2.0, 9.3], 2.0 [95% CI: 1.2, 3.3] and 1.8 [95% CI: 1.2, 2.6]). While these findings are intriguing, the response rate was only 59% (with no evaluation of selection bias) and the paint also contained solvents thought to be associated with spontaneous abortions.

Borja-Aburto et al. (1999) examined the association between blood Pb concentrations and spontaneous abortions in a nested case-control study using incidence density methods and matching for age, calendar time of study entry, public versus private clinic, and gestational age at study entry. They ascertained 668 women during the first trimester of pregnancy in Mexico City. After contacting women biweekly to update pregnancy status, they found 35 cases (6.4%) of spontaneous abortion among women not lost to follow up. An odds ratio of 1.8 (95% CI: 1.1, 3.1) per 5 µg/dL increase in blood Pb was observed after adjustment for spermicide use, active and passive smoking, use of alcohol and coffee, maternal age, education, income, physical activity, hair dye use, use of video display terminals, and medical conditions. Mean blood Pb in cases (12.0 µg/dL, range 3.1-29 µg/dL) was slightly higher than in controls (10.1 µg/dL, range 1.3–26 µg/dL). Further, after categorizing blood Pb into 5 µg/dL intervals, a concentration-response relationship was evident.

More recently, a small study of 57 female workers in a battery plant in China and 62 controls found that 6 spontaneous abortions occurred in the exposed group, compared to none in the controls (Tang and Zhu, 2003). A long-term follow-up of survivors of acute plumbism (Hu, 1991) found increased risk of spontaneous abortions or stillbirths (odds ratio of 1.6 [95% CI: 0.6, 4.0]). Although the study was based on small numbers, the data suggest a persistent association between childhood exposure and outcomes later in life.

A review of eight studies (Borja-Aburto et al., 1999; Driscoll, 1998; Laudanski et al., 1991; Lindbohm et al., 1991a; McMichael et al., 1986; Murphy et al., 1990; Tabacova and Balabaeva, 1993; Taskinen, 1988) evaluating maternal exposure to Pb (blood Pb >30 µg/dL) and spontaneous abortion concluded that there was little evidence that Pb exposure at these relatively high levels was associated with an increased risk in spontaneous abortions (Hertz-Picciotto, 2000). However, Hertz-Picciotto also concluded that methodological difficulties in most of these studies (i.e., small sample sizes, inadequate ascertainment of outcome, and possible residual confounding) limited the confidence in these findings. Further, she noted that exposure in many

of these studies was either measured in an ecologic fashion or biological measures were available, but they were not ascertained during a biologically meaningful period.

Collectively, there is little evidence to support an association between Pb exposure in the female and spontaneous abortion. The only well-designed study which found an association is that of Borja-Aburto et al. (1999); however, these results need to be confirmed in other populations. Studies of spontaneous abortion need be done carefully to avoid possible bias due to recall, use of pregnancies other than the first, and confounding. Retrospective studies, for example, should take full pregnancy histories, including probing for spontaneous abortions versus induced abortions versus stillbirths. In some cultures, for example, induced abortions are frowned upon and women may report spontaneous abortions instead. Additionally, some women may confuse a stillbirth with spontaneous abortion, especially if she is unable to adequately date her pregnancy using date of last menstrual period. Although use of the most recent pregnancy may curtail problems of recall, other concerns dictate that the first pregnancy be used in studies of spontaneous abortion because the risk of subsequent spontaneous abortion depends on the history of spontaneous abortion. Finally, while few variables are known confounders of this relationship, the following should be controlled: maternal age, education and other SES indicators, cigarette smoking, and alcohol use. Several studies of spontaneous abortion did not properly adjust for these potentially confounding variables.

One final concern regards the type of spontaneous abortion. Very early spontaneous abortions, i.e., before a clinical pregnancy is diagnosed, may be missed; assuming, however, that both exposed and unexposed women have the same rates of early spontaneous abortions, this would bias the association towards the null. Indeed, this may be true, as many very early spontaneous abortions may be chromosomally abnormal and probably not attributable to Pb exposure.

6.6.4.2 Spontaneous Abortion and Paternal Exposure to Lead

Three studies evaluated paternal exposure to Pb and spontaneous abortion. Lindbohm et al. (1991a), using national databases to identify pregnancy outcomes among 99,186 births in Finland, found no association between paternal employment in jobs with Pb exposure and spontaneous abortion (odds ratio of 0.9 [95% CI: 0.9, 1.0]). In a follow up case-control study (Lindbohm et al., 1991b), they ascertained paternal exposure status during the period of

spermatogenesis in 213 cases of spontaneous abortion and 500 controls. Exposure was ascertained using blood Pb concentrations measured during spermatogenesis for 6% of men; for the remaining 94%, exposure was estimated using a regression model where the independent variables were blood Pb levels measured either prior to or after the period of spermatogenesis. Blood Pb (either measured or estimated) was not associated with spontaneous abortion. However, when the analysis was restricted to men with measured blood Pb, blood Pb levels >30 µg/dL were associated with an increased odds of spontaneous abortion (odds ratio of 3.8 [95% CI: 1.2, 2.0]); but, this result was based only on 12 cases and 6 controls.

The third study (Alexander et al., 1996b) found no association between men employed in a Pb smelter and spontaneous abortion. For men with "moderate" exposure jobs the estimated odds ratio was 0.8 (95% CI: 0.5, 1.5) and for those with "high" exposure jobs, the estimated odds ratio was 1.4 (95% CI: 0.7, 2.5). Further when blood Pb 1 year prior to the pregnancy was used as the exposure measure, no increased odds of spontaneous abortion were found. These results, however, are based on a low participation rate in eligible workers (37%) and should be interpreted with caution. Overall, the available studies provide little evidence for an association between Pb exposure in the male and spontaneous abortions.

6.6.5 Fetal Growth

The results of epidemiologic studies regarding the association between Pb exposure and birth weight are inconsistent. Cross-sectional studies (Clark, 1977; Gershanik et al., 1974; Moore et al., 1982; Rajegowda et al., 1972) did not find significant correlations between blood Pb and birth weight, nor did a study using placental Pb as the exposure variable (Wibberley et al., 1977). A case-control study (Bogden et al., 1978) comparing 25 low birth weight babies (1,500–2,500 grams) to 25 controls (>2,500 grams) matched on maternal age, race and social class found a small, nonsignificant difference in maternal and cord blood Pb levels. Mean maternal blood Pb concentrations were 16.2 ± 4.5 µg/dL and 15.3 ± 5.2 µg/dL and mean cord blood Pb levels were 13.8 ± 4.4 µg/dL and 13.1 ± 4.3 µg/dL in cases and controls, respectively. A further study (Huel et al., 1981) found no differences in maternal and fetal hair Pb levels between infants born small-for-gestational-age compared to those of normal birth weight.

In 1984, Needleman et al. (1984) reported on a cross-sectional study of 5,183 births of at least 20 weeks gestation in Boston, MA. No associations were found between the proportion of

births under 2,500 grams and cord blood Pb. Exposure levels in this study were relatively low for the time; cord blood Pb ranged from <1 to 35 µg/dL. A reanalysis of these data found no relationship between cord blood Pb and birth weight when birth weight was considered as a continuous variable (Bellinger, et al., 1991). However, when birth weight was categorized as low birth weight (<2,500 grams), small for gestational age (<10th percentile for gestational age), or intrauterine growth retarded (>2 standard deviations below the mean for gestational age), relative risks of 1.6 (95% CI: 1.0, 2.6), 1.2 (95% CI: 0.8, 1.6) and 1.9 (95% CI: 1.0, 3.4), respectively, were found for each 10 µg/dL increase in cord blood Pb levels. Increased relative risks also were found for cord blood Pb levels ≥15 µg/dL, compared to cord blood Pb levels <15 µg/dL; however, only 83 of the 5,183 women had exposures in the high range, resulting in imprecise estimates. These data suggest that Pb-related modest reductions in birth weight are perhaps plausible when birth weight is expressed as a function of gestational age.

The prospective study of Pb exposure in and around Port Pirie, Australia (McMichael et al., 1986) followed 749 pregnancies of at least 20 weeks duration. Mean maternal blood Pb levels at mid-pregnancy were 10.1 µg/dL and 7.0 µg/dL for women residing in Port Pirie and the surrounding communities, respectively. After excluding 9 sets of twins and 10 cases for which the maternal last menstrual period could not be ascertained, no relationship was found between either cord blood Pb or maternal blood Pb measured at mid-pregnancy or at delivery and birth weight in a multivariate regression model controlling for known determinants of birth weight.

A prospective study in two towns in Kosovo, Yugoslavia evaluated relationships between birth weight (adjusted for gestational age using last menstrual period) and (a) maternal blood Pb at mid-pregnancy and delivery and (b) cord blood Pb (Factor-Litvak et al., 1991). The towns were vastly different in exposure patterns, as one was the site of a Pb smelter, refinery and battery plant (n = 401, mean mid-pregnancy blood Pb 19.0 µg/dL) and one was relatively unexposed (n = 506, mean mid-pregnancy blood Pb 5.6 µg/dL). No associations were found between any of the biomarkers of Pb exposure and birth weight in either crude analyses or analyses adjusted for potentially confounding variables.

While the aforementioned studies generally found no association between environmental Pb exposure and birth weight, three other studies have shown large reductions in birth weight related to Pb exposure. These studies, however, have questionable study designs. Nordström et al. (1978b, 1979) in a series of ecologic analyses known as the Swedish Smelter Study, found

significant reductions in birth weight between the offspring of women either working at or living in close proximity to the smelter. The 125 gram deficit in birth weight among the offspring of women living closest to the smelter was confined to those with parity of three or more, an observation which does not appear to be biologically plausible. Moreover, the ecological nature of the study did not allow for individual measurements of blood Pb or for control of potentially confounding variables. Hence, while suggestive, these data do not provide strong evidence for a causal association between Pb exposure and birth weight.

In a cross-sectional study of 100 "normal" singleton births, a negative correlation was found between placental Pb concentration and birth weight (Ward et al., 1987). Mean placental Pb levels in 21 infants weighing less than 3,000 grams were 2.35 ± 0.9 µg/g compared to 1.12 ± 0.4 µg/g in 10 infants weighting more than 4,000 grams. This study has several limitations. First, no statistical adjustment was made for multiple comparisons (many exposures were studied). Second, potentially confounding variables were not controlled. Third, only 31 of the 100 infants, representing the extremes of the birth weight distribution, were studied. Hence, this study also does not provide strong evidence for an association.

In Cincinnati, OH, the association between Pb exposure and birth weight was examined in offspring of a cohort of young (mean maternal age 22.7 years) inner city women, 85% being African-American and 86% being on public assistance, with a mean IQ of 75 (Dietrich et al., 1987a). The mean gestational period of the neonates, as determined by physical examination, was 39.5 weeks. A decrement in birth weight of 172 grams was associated with an increase in blood Pb from 10 to 30 µg/dL. Lead exposure in this group was relatively low with a mean blood Pb of 8.0 ± 3.7 µg/dL. In a sample of women from this cohort, the interaction between blood Pb and maternal age was significantly associated with birth weight; the effect varied from a decrease of 64 grams for 18 year old mothers to 660 grams for 30 year old mothers, as blood Pb rose from 10 to 30 µg/dL (Bornschein et al., 1989). Although the Cincinnati study is highly suggestive of an effect (especially an effect which varies by maternal age) three factors should be considered in the interpretation of their findings. First, length of gestation was estimated by examining the neurological and physical maturity of the neonate (Ballard et al., 1979); other investigators find assessment of gestational age using this scale overestimates gestational age in preterm infants (Constantine et al., 1987; Kramer et al., 1988; Shukla et al., 1987; Spinnato et al., 1984). Second, it is possible that the association between Pb and birth weight differs by maternal

characteristics such as race, ethnicity, and SES; however, no study has provided a population sufficiently heterogeneous to examine this possible source of difference. Finally, it is possible that confounding by unmeasured maternal lifestyle characteristics may account for the reported association.

A hospital-based study of cord blood Pb and pregnancy outcomes in Quebec, Canada, between June 1993 and January 1995 found a slight increase in cord blood Pb levels among infants with birth weight <2,500 grams (Rhainds et al., 1999). For those infants with birth weight <2,500 grams, the geometric mean blood Pb was 1.8 µg/dL (95% CI: 1.6, 2.9) compared to 1.6 µg/dL (95% CI: 1.5, 1.7), 1.6 µg/dL (95% CI: 1.5, 1.7), and 1.5 µg/dL (95% CI: 1.5, 1.6) among those with birth weights 2,500-2,990, 3,000-3,499, and ≥3,500 grams, respectively. Although suggestive, the study did not control for potentially confounding variables. Also mean levels of measured cord blood levels of mercury and organochlorine compounds were higher as well in infants who weighed <2,500 g.

More recently, Irgens et al. (1998) using data from the Norwegian birth registry found that women occupationally exposed to Pb (none/low compared to moderate/high) were more likely to deliver a low birth weight infant (odds ratio of 1.3 [95% CI: 1.1, 1.6]). No association was found for paternal occupational Pb exposure. Parental occupational exposure to Pb was not associated with low birth weight in the Baltimore-Washington Infant Study database (Min et al., 1996), although subgroup analysis suggested that high paternal exposure may be associated with small-for-gestational-age infants (odds ratio of 2.9 [95% CI: 0.9, 9.2]). Similar findings were reported by Lin et al. (1998) who compared offspring of Pb-exposed workers with those of bus drivers. No associations were reported between Pb exposure and low birth weight except among the group of men with blood Pb levels >25 µg/dL for over 5 years (relative risk of 3.4 [95% CI: 1.4, 8.4]).

Using bone Pb as the metric of exposure, González-Cossio et al. (1997) found associations between tibia bone Pb (but not patella bone Pb or umbilical cord blood Pb) and reduced birth weight. Bone Pb was measured one month after delivery. Infants with tibia bone Pb in the highest quartile (≥15.15 µg Pb/g bone mineral) were, on average, 156 g lighter than those in the lowest quartile (≤4.50 µg Pb/g bone mineral). Further analyses of these data (Hernandez-Avila et al., 2002) found an association between infants in the highest quintile of tibia bone Pb and shorter birth length (odds ratio of 1.8 [95% CI: 1.1, 3.2]).

Two studies have evaluated relationships between Pb exposure and head circumference. In one study (Hernandez-Avila et al., 2002), among 233 women in Mexico City, high maternal patella bone Pb was associated with increased risk of a low head circumference score at delivery (1.02 per µg Pb/g bone mineral [95% CI: 1.01, 1.04]). Similar findings were reported by Rothenberg et al. (1999) who found a reduction in six-month head circumference of 1.9 cm (95% CI: 0.9, 3.0) as maternal blood Pb rose from 1 to 35 µg/dL. This study, however was plagued by multiple comparisons, as head circumference was measured nine times and prenatal blood Pb six times—with only one statistically significant result being found.

Potential confounders need to be adjusted for to properly assess the relationship between Pb exposure and fetal growth. Factors consistently associated with fetal growth include gender, ethnic origin, maternal body build (i.e., pre-pregnancy weight, height), parity, SES, gestational weight gain and nutritional intake during pregnancy, maternal illness, and cigarette smoking (Kramer, 1987). Factors with less established associations include alcohol consumption (Kline et al., 1987; Kramer, 1987) and street drug use (Kline et al., 1987; Kramer, 1987; Zuckerman et al., 1989). To the extent that these factors are associated with blood Pb as well as with fetal growth, they must be accounted for in the analysis.

A further problem pertains to the measure of exposure used in most of these studies. Blood Pb concentration reflects relatively recent (i.e., in the past 90 days) exposure; thus it does not reflect exposure over the mother's lifetime. Indeed, there is some evidence suggesting that bone Pb, a measure of cumulative exposure, may be mobilized during pregnancy (Silbergeld, 1991). A single blood Pb measure will not reflect such mobilization, particularly if mobilization is not constant over the course of pregnancy. Thus, in all studies, excepting that of González-Cossio et al. (1997), exposure may be misclassified. The effect of such misclassification will be to strengthen the findings of studies which support the null hypothesis. For those studies which find an association between blood Pb concentration and fetal growth, the inference would be to higher exposure levels. It is difficult to examine the extent of this misclassification as no studies have sufficient numbers of serial blood Pb measures to estimate the variation during pregnancy.

Studies to date are inconsistent regarding the association between Pb exposure and birth weight. Several large prospective studies found no association (Factor-Litvak et al., 1991; McMichael et al., 1986), while at least one (Bornschein et al., 1989) did find an association in specific subgroups of women. However, there is limited evidence (Bellinger et al., 1991) for an

association between Pb exposure and low birth weight (i.e., <2,500 g), small for gestational age (i.e., <10th percentile for gestational age), and intrauterine growth retardation (i.e., >2 standard deviations below the mean for gestational age). These prospective studies were all well-conducted, adequately measured exposure and outcome, and controlled for potential confounding variables. They did, however, take place in very different populations, suggesting that the association between Pb and fetal growth may depend on the population being studied.
The Yugoslavia study (Factor-Litvak et al., 1991) took place in two towns in Kosovo, Yugoslavia, which were divergent on exposure and somewhat comparable on other variables. The Port Pirie study took place in a middle class area of Australia (McMichael et al., 1986). The Boston study (Bellinger et al., 1991) took place across a range of social strata in Boston; the exposure in the highest social group was attributable to renovation of older housing stock. Finally, in the Cincinnati study (Bornschein et al., 1989), the study sample was comprised of lower social class African Americans and the mean IQ of the mothers was 75. It is possible that in this latter study, there was some unmeasured variable which accounts for the observed interaction. Thus, the evidence suggests at most a small effect of Pb exposure on birth weight and possibly a small association between Pb exposure and several dichotomized measures of fetal growth.

6.6.6 Preterm Delivery

Early evidence regarding an association between environmental Pb exposure and preterm delivery was inconsistent. In 1976, Fahim et al. found a preterm delivery rate of 13% in 254 pregnant women living near a Pb mining community in Missouri, compared to 3% in 249 women living in a control location. These investigators also found higher concentrations of Pb in amniotic membrane, but not higher placental or cord Pb in preterm compared to term deliveries, regardless of the women's residential locale. This observation prompted other studies of Pb and preterm delivery.

Of the cross-sectional studies, the three which show no association employed cord blood Pb as the exposure measure and restricted gestational age (Angell and Lavery, 1982; Bellinger et al., 1991; Needleman et al., 1984; Rajegowda et al., 1972). In contrast, three other studies used different exposure markers (placental Pb, maternal and cord blood Pb, and maternal and fetal hair Pb) and found statistically significant associations (Huel et al., 1981; Moore et al.,

1982; Ward et al., 1987). Other studies evaluated pregnancy outcomes in relation to maternal delivery blood Pb (McMichael et al., 1986; Rahman and Hakeem, 2003).

Of the prospective studies, the Cincinnati study (Bornschein et al., 1989) found no association between both maternal blood Pb at mid-pregnancy or maternal blood Pb during the neonatal period (10 days post delivery) and preterm delivery. However, gestational age was estimated by examining the neurological and physical maturity of the neonates (which tends to overestimate gestational age) and not actual dates. In Port Pirie, Australia (McMichael et al., 1986), a concentration-response relationship between maternal delivery blood Pb and preterm delivery was reported. Odds ratios ranged from 2.1 to 4.4 in women with blood Pb of 7.7 to 10.6 μg/dL and >13.5 μg/dL, respectively, compared to those with blood Pb <7.7 μg/dL. Savitz et al. (1990) used data from the National Natality Survey and found an odds ratio of 2.3 (95% CI: 0.7, 7.0) between maternal occupational exposure to Pb and preterm delivery; however, the estimated odds ratio was based on only 7 cases. In the Yugoslavia study (Factor-Litvak et al., 1991), no associations were found between cord blood Pb or blood Pb measured at mid pregnancy or delivery and either preterm delivery (defined as delivery <37 completed weeks) or gestational age. A registry study in Norway (Irgens et al., 1998) which linked births between 1970 and 1993 to census-based occupation records found a slightly increased odds of preterm delivery among moderate/high Pb-exposed women, compared to those with no or low exposure (odds ratio of 1.13 [95% CI: 0.98, 1.29]). Paternal exposure was not found to increase the risk of preterm birth.

An ecologic study in Canada (Philion et al., 1997) examined 30 years of birth records, corresponding to 9,329 births in a smelter city and a control city. Outcome variables were intrauterine growth retardation defined as small for gestational age. The odds ratio for intrauterine growth retardation in the smelter city compared to the control city was 0.83. Further analysis, stratifying time into 5-year intervals also revealed no associations.

A case control study in Mexico City (Torres-Sánchez et al., 1999) evaluated 161 preterm births and 459 full term births. Cord blood Pb was significantly higher in the preterm group (9.8 ± 2.0 μg/dL) compared to the full term group (8.4 ± 2.2 μg/dL) only among primiparous women.

Using data from the Baltimore-Washington Infant Study database, Min et al. (1996) found a small association between paternal occupational exposure in the high range and preterm

delivery with appropriate weight for gestational age (odds ratio of 2.1 [95% CI: 0.7, 6.5]) and preterm delivery with small for gestational age (odds ratio of 2.4 [95% CI: 1.9, 3.1]). Similar findings were reported by Lin et al. (1998). Comparing the offspring of Pb-exposed workers with those of bus drivers, they found an elevated relative risk for preterm delivery (3.0 [95% CI: 1.6, 6.8]) only among men with blood Pb >25 µg/dL for over 5 years.

In contrast to fetal growth, few factors are consistently related to preterm delivery; thus in both developed and developing countries the majority of preterm deliveries remain unexplained (Kramer 1987; Van Den Berg and Oechsli, 1984). Factors which are inconsistently associated with preterm delivery include maternal age, SES, pre-pregnant weight, prior history of preterm delivery or spontaneous abortion, and cigarette smoking (Kline et al., 1987; Kramer, 1987). Thus, these factors must be evaluated as potentially confounding factors in studies of Pb exposure and preterm delivery.

For preterm delivery, or reduced length of gestation, the evidence for an association with Pb exposure is contradictory. Several of the prospective studies found no evidence of an association (Bornschein et al., 1989; Factor-Litvak et al., 1991), whereas one found a concentration-response relationship (McMichael et al., 1986). Further, two well-done registry studies (Irgens et al., 1998; Savitz et al., 1990) found some evidence of an association, albeit the number of exposed cases was small. It seems unlikely that the association between Pb exposure and preterm delivery is large, but, more research is clearly necessary.

6.6.7 Congenital Abnormalities

Needleman et al. (1984) found an association between cord blood Pb and minor congenital anomalies among 4,354 infants born in a single hospital in Boston, MA. All data were obtained from hospital records, not from direct examination of the infants. The most common anomalies were hemangiomas, lymphangiomas, minor skin problems (tags and papillae), and undescended testicles. Blood Pb levels were not found to be associated with individual anomalies.

More recently, a number of studies have considered parental Pb related to occupational exposure and risk of congenital anomalies in the offspring. In Finland, Sallmén et al. (1992) evaluated the associations between congenital malformations and paternal exposure during the time of spermatogenesis. The overall estimated unadjusted odds ratio for men with blood Pb

levels >20 µg/dL was 2.4 (95% CI: 0.9, 6.5). Due to small sample sizes, the investigators could only adjust for one potentially confounding factor at a time; this resulted in odds ratios ranging from 1.9 to 3.2. Of note is the lack of consistency of malformations among the five men with the highest blood Pb. The malformation observed included congenital heart disease, oral cleft, club foot, polydactyly, and anomalies of the adrenal gland. The breadth of these anomalies suggests either that Pb affects physical development throughout gestation or that this association represents a chance finding. Among 2,021 pregnancies, Alexander et al. (1996b) found slightly elevated odds ratios for congenital defects among men in the Pb smelting industry with moderate exposure (odds ratio of 1.9 [95% CI: 0.6, 6.3]) and high exposure (odds ratio of 2.7 [95% CI: 0.7, 9.6]). These estimates are based on 30 birth defects and 12 stillbirths. No analyses were presented which considered individual birth defects. In Norway, neither maternal (odds ratio of 1.25 [95% CI: 0.8, 1.9]) nor paternal (odds ratio of 0.94 [95% CI: 0.8, 1.1]) occupational Pb exposure was associated with serious birth defects (Irgens et al., 1998). Similar results were reported by Kristensen et al. (1993) between paternal Pb exposure and birth defects, with the exception of a fourfold increase in the risk of cleft lip among male offspring.

The risk of parental Pb exposure and neural tube defects was evaluated in a case-control study of 88,449 births (363 neural tube defects) over a 25-year period in Fylde, England (Bound et al., 1997). Women living in areas in which the water Pb concentration was >10 µg/L were more likely to deliver a child with a neural tube defect. The association was consistent for anencephaly (n = 169) and spina bifida/cranium bifidum (n = 195), even after adjusting for social class. These authors posit that the association could be a direct effect of Pb on neural tube closure or an indirect effect, the latter meaning a reduction in uptake of zinc (due to Pb exposure) leading to a reduction in folate uptake. Irgens et al. (1998) partially confirmed these effects on neural tube defects in mothers occupationally-exposed to Pb (relative risk of 2.87 [95% CI: 1.05, 6.38]), but not for paternal Pb exposure.

The association between total anomalous pulmonary venous return and parental Pb exposure during pregnancy (self reported, obtained from industrial hygiene measures, or from a job exposure matrix) was examined in the Baltimore-Washington Infant Study (Jackson et al., 2004). In this case-control study, maternal periconceptional (i.e., 3 months prior to conception through the first trimester) exposure to Pb resulted in an estimated odds ratio of 1.57 (95% CI: 0.64, 3.47). For Pb-exposed men, the estimated odds ratio was 1.83 (95% CI: 1.00, 3.42).

Findings from this study support a possible association between paternal Pb exposure and total anomalous pulmonary venous return**.**

Taken together, the evidence suggests few associations between periconceptional or prenatal exposure to Pb and congenital anomalies. There is a suggestion of small associations with high levels of exposure, but many of those studies relied on occupational histories rather than on actual measures of blood Pb levels.

6.6.8 Summary of the Epidemiologic Evidence for the Reproductive and Developmental Effects of Lead

Overall, since the 1986 Lead AQCD, a substantial body of work has evaluated the associations between Pb exposure and reproductive outcomes. It is now clear that Pb clearly crosses the placenta during all trimesters and maternal exposure results in fetal exposure. For many other outcomes, the observed associations are relatively small, especially at the levels of exposure that are currently of interest.

Further, there may be populations with increased fetal susceptibility, including populations with high rates of smoking and alcohol use, those using ethnic remedies and cosmetics, and those who use Pb glazed pottery. Low levels of calcium intake may also increase fetal exposure.

- The available evidence suggests small associations between exposure to Pb and male reproductive outcomes. These include perturbed semen quality and increased time to pregnancy. These associations appear at blood Pb levels >45 µg/dL, as most studies only considered exposure in the occupational setting. More research is needed regarding possible male reproductive effects at exposure levels in the lower (and currently more relevant) range. There are no adequate data to evaluate associations between Pb exposure and female fertility.

- With one exception, there is no evidence to suggest an association between either maternal or paternal Pb exposure and increased risk of spontaneous abortions. One study in Mexico where the mean maternal blood Pb levels were in the moderate range (i.e., 10-12 µg/dL) suggests an association.

- To date, the evidence suggests at most a small association of Pb exposure with birth weight, fetal growth, preterm delivery, and congenital anomalies. The reviewed studies occurred in very different populations, and the small associations may reflect some unmeasured or unknown confounding variable.

6.7 GENOTOXIC AND CARCINOGENIC EFFECTS OF LEAD

6.7.1 Summary of Key Findings from the 1986 Lead AQCD

The 1986 EPA Lead AQCD reviewed five epidemiologic studies of occupationally exposed workers (Cooper and Gaffey, 1975; Davies, 1984; Selevan et al., 1985; Sheffet et al., 1982; McMichael and Johnson, 1982). These workers were exposed to inorganic Pb compounds such as Pb oxides and Pb sulfides. The EPA noted that Cooper and Gaffey reported a significant increase in lung and gastrointestinal cancer among battery and smelter workers in the United States (standardized mortality ratios of 1.50 and 1.48 respectively among smelter workers, and 1.32 and 1.23 among battery workers). Further, much of this exposure was by inhalation and ingestion of Pb oxides, which are relatively insoluble, adding some plausibility to the occurrence of cancer at these two sites. Sheffet et al. (1982) found a nonsignificant excess of stomach cancer among U.S. Pb chromate pigment workers. However, Davies (1984) did not find any cancer excess among U.K. Pb chromate pigment workers. The EPA noted that Selevan et al. (1985) found a significant excess of kidney cancer among U.S. Pb smelter workers based on 6 cases. This finding was judged striking because it mimicked the findings of kidney cancer in animals. The EPA judged that the McMichael and Johnson (1982) study of Pb-poisoned workers was not particularly informative because the non-poisoned workers may have had substantial Pb exposure and no details were given on how Pb poisoning was determined. In summary the EPA felt the evidence was insufficient, stating that "little can now be reliably concluded from available epidemiologic studies."

The studies by Cooper and Gaffey (1975) and Selevan et al. (1985), which are both important because they are large occupational cohorts with documented high exposure, have been updated and are further reviewed below. A cohort study of U.K. battery workers (Malcolm and Barnett, 1982) is also reviewed below.

EPA in 1986 also presented data on human cytogenetic studies, reproducing data from an earlier 1980 International Agency for Research on Cancer (IARC) monograph for metals and metallic compounds (IARC, 1980). For Pb, 10 chromosomal aberration studies were judged to be "positive" and 6 such studies were judged to be "negative." On the whole, the EPA considered that "under certain conditions Pb compounds are capable of inducing chromosomal aberrations in vivo and in tissue cultures." The EPA also reviewed more limited data from two

human studies of sister chromatid exchange (Dalpra et al., 1983; Grandjean et al., 1983), one of which was positive and one negative.

6.7.2 Summary of Key Findings by the International Agency for Research on Cancer and the National Toxicology Program

IARC reviewed inorganic and organic Pb compounds in its monograph number 87 in February of 2004 (IARC, 2005), and concluded that inorganic Pb compounds were probable human carcinogens (Group IIA). The IARC classification of inorganic Pb compounds as probable human carcinogens was based on limited evidence in humans and sufficient evidence in animals. Regarding organic Pb compounds (e.g., tetraethyl Pb), IARC concluded that there was insufficient information to make any judgment.

Regarding the human studies, IARC based its evaluation largely on six occupational cohort studies of highly-exposed workers, which were felt to be particularly informative (battery workers in the United States and the United Kingdom, smelter workers in Italy, Sweden, and the United States). The IARC assessment focused on four cancer sites: lung, stomach, kidney, and brain. IARC noted that lung showed a significant elevation in one study (Lundström et al., 1997) and nonsignificant elevations in a number of others. However, the significant elevation of lung cancer in Lundström et al. appeared to be inextricably associated with arsenic in addition to Pb exposure (Englyst et al., 2001). IARC concluded that the strongest epidemiologic evidence for Pb carcinogenicity was for stomach cancer, noting that four cohort studies showed a consistent 30-50% excess of stomach cancer versus external referent populations. IARC noted that confounding by ethnicity, diet, *Helicobacter pylori* infections, or SES could have played a role in the stomach cancer excesses. Finally, IARC noted that while one cohort study showed a 2-fold excess of renal cancer (Steenland et al., 1992), the other studies showed no excess. Similarly, there were no consistent excesses of brain cancer, although one study did find a significant positive dose-response between glioma and blood Pb levels, based on small numbers (Anttila et al., 1996).

The National Toxicology Program (NTP) in 2003 evaluated the carcinogenicity of Pb and Pb compounds. A summary of its evaluation can be found in NTP's Report on Carcinogens (NTP, 2004), and the detailed evaluation is also available (NTP, 2003). NTP, like IARC, concluded that "Pb and Pb compounds are reasonably anticipated to be human carcinogens based

on limited evidence from studies in humans and sufficient evidence from studies in experimental animals." The NTP considered that "the strongest epidemiologic evidence was for lung and stomach cancer, which are consistently but weakly associated with occupations and industries entailing Pb exposure and with indices of individual Pb exposure, including job history and biological monitoring of occupationally exposed and general populations. However, most studies of Pb exposure and cancer reviewed had limitations, including poor exposure assessment and failure to control for confounders (other factors that could increase the risk of cancer, including lifestyle factors and concurrent occupational exposure to other carcinogens), and did not demonstrate relationships between the amount of exposure (concentration or duration, for example) and the magnitude of cancer risk." NTP, like IARC, also relied heavily on occupational cohort studies in its evaluation of the epidemiologic evidence. NTP (2003) noted that "the mechanisms by which Pb causes cancer are not understood. Lead compounds do not appear to cause genetic damage directly, but may do so through several indirect mechanisms, including inhibition of DNA synthesis and repair, oxidative damage, and interaction with DNA-binding proteins and tumor-suppressor proteins."

Both the IARC and NTP evaluations of human evidence relied primarily on occupational studies of highly exposed workers, in which limited evidence of stomach and to some extent lung carcinogenicity was found. There are seven such studies with relatively large populations (Anttila et al., 1995; Carta et al., 2005; Fanning, 1988; Gerhardsson et al., 1995; Lundström et al., 1997; Steenland et al., 1992; Wong and Harris, 2000). A further study (Ades and Kazantzis, 1988) also addresses Pb exposure in a large occupational cohort, albeit compromised by the strong correlation between arsenic and Pb exposure in the cohort. It should be noted that the blood Pb levels among these workers were generally three to five times higher than blood Pb levels in the two studies of the general U.S. population (Jemal et al., 2002; Lustberg and Silbergeld, 2002; both based on NHANES II) with environmental exposures. For example, mean blood levels in two studies of U.S. Pb smelter workers averaged 56 µg/dL in Steenland et al. (1990) in 1976 and 80 µg/dL in Cooper et al. (1985) during the period 1947-1972, while the U.S. population enrolled in NHANES II in late 1976-1980 averaged 14 µg/dL. General population blood Pb levels have decreased markedly since the 1970s in many industrial countries with the banning of leaded gasoline. U.S. general population levels in the early 1990s thus averaged 3 µg/dL according to NHANES III (ATSDR, 1999; see Lead Toxicological Profile,

page 409). Regarding the occupational studies, while exposure is well documented, detailed exposure-response data are generally not available, precluding quantitative inference about likely effects in low exposure groups based on these studies. The high exposure occupational cohorts are the most informative for deciding whether Pb is likely to cause cancer, simply because high doses are more likely to show detectable effects than low doses, if effects exist. If Pb does cause cancer, and assuming there is no threshold below which exposure does not cause cancer, current low level exposures among the general public may produce some level of Pb-related cancers due to the potential exposure of a large number of people.

6.7.3 Genotoxicity of Lead

The NTP reviewed in some detail the genotoxicity studies over the period 1970–2002. These studies are cross-sectional studies, mostly of occupationally exposed workers compared to a control population. Usually blood Pb levels are available to document exposure. Outcomes consisted of chromosomal aberrations (CA), sister chromatid exchange (SCE), micronuclei formation (MN), and studies of DNA damage (often via the comet assay) and/or measures of the mitotic activity. Of these outcomes, only CAs have been shown to have a positive relationship to subsequent cancer (Hagmar et al., 2004, Rossner et al., 2005). SCEs are generally considered a marker of exposure to environmental agents which affect DNA, but do not necessarily predict cancer risk. MN and DNA damage are thought to indicate genotoxicity with unknown effect on cancer risk. The informativeness of these outcomes regarding possible human carcinogenicity of Pb are thus clearly secondary to direct information on cancer risk from epidemiologic studies.

Since the NTP review, there have been three additional cytogenetic studies which are informative regarding Pb (Palus et al., 2003, Minozzo et al., 2004, and Fracasso et al., 2002), as well as one mutation study (Van Larebeke et al., 2004). As detailed in Annex Table AX6-7.1, all four of these studies (two of DNA damage, one of MN, and one of a specific mutation frequency) were positive in significantly linking Pb exposure to the outcome. Treatment of potential confounding factors varied across studies, but there was no indication that more extensive adjustment for such factors was associated with weaker relationships between Pb exposure and genotoxic endpoints. Potential coexposure to other potentially genotoxic metals remains an issue, although Palus et al. (2003) found as much or more evidence of genotoxicity

for each major endpoint examined among heavily Pb-exposed workers as among those expected to have the heaviest exposure to Cd.

The results of the four most recent studies as well as those reviewed by the NTP are summarized in Table 6-3. Of eleven studies of chromosomal aberrations (CA), six were judged to show a positive relationship between CA and Pb, four were judged negative, and one was neither clearly positive nor negative. In general, these studies were done in the 1970s and 1980s; only one dates from the 1990s. There were nine studies of sister chromatid exchange. Of these, four were judged positive, three negative, and two could not be judged clearly one way or the other. It is notable that the positive studies were generally the most recent. There were four MN studies, all of which were judged positive. Finally, there were nine studies of DNA damage and/or mitotic activity. These varied in the specific outcome, although many used a comet assay to measure oxidative damage to DNA. Eight of these nine studies were judged positive in the sense that increased DNA damage or mitotic activity was related to Pb exposure, while one was judged negative.

Table 6-3. Results of Epidemiologic Studies on the Genotoxicity of Lead Exposure[a]

	Results		
Studied Outcome	**Positive**	**Mixed**	**Negative**
Chromosomal Aberrations (CA)	6	1	4
Sister Chromatid Exchange (SCE)	4	2	3
Micronucleus Formation (MN)	5	0	0
DNA Damage/Mitosis	10	0	1
Gene Mutation	1	0	0

[a] Results summarize the overall findings of epidemiologic studies addressing the potential genotoxic effects of lead exposure. Some studies addressed multiple aspects of genotoxicity; for these studies, their results for each of the listed categories of genotoxic outcomes are presented separately.

While the overall the evidence from cytogenetic studies is mixed, more recent studies which were focused on DNA damage or mitotic activity have tended to be largely positive. However, it is not known whether these outcomes predict subsequent cancer risk.

6.7.4 Meta-analyses of Lead and Cancer

There have been two published meta-analyses of the carcinogenicity of Pb and Pb compounds. Their major findings are summarized in Table 6-4. Steenland and Boffeta (2000) relied on eight occupational cohort studies of highly-exposed workers (seven cohort studies, one nested case-control), all of which had documentation of exposure levels. Meta-analyses were conducted for lung, stomach, kidney, and brain cancer. The combined lung cancer relative risk was 1.30 (95% CI: 1.15, 1.46), based on 675 lung cancer deaths. However, the authors noted that the lung cancer findings were not consistent across studies, and were influenced highly by one study (Lundström et al., 1997) in which confounding by arsenic was likely. Exclusion of this study dropped the combined lung cancer relative risk to 1.14 (95% CI: 1.04, 1.73). The strongest positive evidence was for stomach cancer (relative risk 1.34 [95% CI: 1.14, 1.57], 181 observed deaths). There was little positive evidence for renal cancer (relative risk 1.01 [95% CI: 0.72, 1.42], 40 deaths), or brain cancer (relative risk 1.06 [95% CI: 0.81, 1.40]). All meta-analyses used fixed effects models, given that no evidence of heterogeneity was found across studies (as long as Lundström et al.'s lung cancer results were excluded).

Table 6-4. Results of Meta-Analyses Addressing the Association Between Lead Exposure and Cancer

	Risk Estimate (95% CI) for Indicated Outcome [Number of Studies Utilized in Estimate]		
Meta-Analysis	**Lung Cancer**	**Stomach Cancer**	**Renal Cancer**
Fu and Boffetta (1995)	1.24 (1.16, 1.33) [n = 15]	1.33 (1.18, 1.49) [n = 10]	1.19 (0.96, 1.48) [n = 5]
Fu and Boffetta (1995)	1.42 (1.05, 1.92) [Battery/smelter only]	1.50 (1.23, 1.83) [Battery/smelter only]	1.26 (0.70, 2.26) [Battery/smelter only]
Steenland and Boffetta (2000)	1.30 (1.15, 1.46) [n = 8 – cohort only]	1.34 (1.14, 1.57) [n = 8 – cohort only]	1.01 (0.72, 1.42) [n = 7 – cohort only]

Fu and Boffetta (1995) conducted an earlier meta-analysis in which they reviewed 16 cohort and 7 case-control studies. Different numbers of studies were used for meta-analyses of different outcomes, dependent on whether that outcome was reported separately, among other factors. Twelve occupational studies were used in a meta-analysis of lung cancer, resulting in a combined relative risk of 1.29 (95% CI: 1.10, 1.50) (random effects model, reflecting significant heterogeneity of lung cancer results across studies). Meta-analyses using fixed effects (no significant heterogeneity between studies) resulted in relative risks of 1.33 (95% CI: 1.18, 1.49) for stomach cancer (10 studies), of 1.19 (95% CI: 0.96, 1.48) for kidney cancer (5 studies), and 1.41 (95% CI: 1.16, 1.71) for bladder cancer (5 studies). No meta-analysis was conducted for brain cancer. Restricting analyses for stomach, lung, and kidney cancer to those studies with the highest occupational exposure to Pb (3 to 5 studies of battery and smelter workers) resulted in slightly higher relative risks. The authors concluded that "the findings from the workers with heavy exposure to Pb provided some evidence to support the hypothesis of an association between stomach and lung cancer and exposure to Pb. The main limitation of the present analysis is that the excess risks do not take account of potential confounders, because little information was available for other occupational exposures, smoking, and dietary habits. The excess risk of stomach cancer may also be explained, at least in part, by nonoccupational factors. For bladder and kidney cancers, the excess risks are only suggestive of a true effect because of possible publication bias."

6.7.5 Review of Specific Studies on the Carcinogenicity of Lead Since the 1986 Lead AQCD

6.7.5.1 Introduction

Many epidemiologic studies of Pb exposure and cancer have been conducted in the past two decades. The most relevant studies focus on exposure via occupational sources, wherein the most intense exposure to Pb can be expected to occur. This exposure predominantly involves inorganic Pb species. Relevant studies are discussed below, beginning with the most key occupational and general population studies, followed by a brief summary of other relevant studies.

6.7.5.2 Key Studies of Occupational Populations in the United States

The strongest evidence in the key occupational studies linking Pb exposure to human cancers is that for cancers of the lung and those of the stomach. Of seven large occupational cohort studies available (Ades and Kazantzis, 1988; Anttila et al., 1995; Carta et al., 2005; Gerhardsson et al., 1995; Lundström et al., 1997; Steenland et al., 1992; Wong and Harris, 2000), all showed results consistent with an increase in lung cancer risk among Pb-exposed workers, and in four of these studies the association was statistically significant. Details of these studies are summarized in Annex Table AX6-7.2. A few of these studies are discussed below.

Steenland et al. (1992) followed up 1,990 male U.S. Pb smelter workers, employed from 1940 to 1965, through 1988. Standardized mortality ratios indicated an excess of lung, stomach, kidney, and bladder cancer that did not reach statistical significance. Focusing on workers classified as highly Pb-exposed based on air monitoring records yielded a significant excess for kidney cancer (standardized mortality ratio of 2.39 [95% CI: 1.03, 4.71]), although it did not appear to increase with duration of exposure. Estimates for the other cancers (standardized mortality ratio of 1.11 [95% CI: 0.82, 1.47] for lung; 1.28 [95% CI: 0.61, 2.34] for stomach; 1.33 [95% CI: 0.48, 2.90] for bladder) showed little change with restriction to the high-exposure group. While neither arsenic nor Cd exposure could be controlled for, 1975 NIOSH monitoring data indicated less intense exposure to airborne Cd or arsenic than to Pb. Lead averaged 3.1 mg/m^3 and arsenic 14 $\mu g/m^3$, compared to current OSHA standards of 0.05 mg/m^3 for Pb and 10 $\mu g/m^3$ for arsenic. No data on workers' smoking status were available.

Wong and Harris (2002) extended follow-up on the battery and smelter worker cohort previously reported on by Cooper et al., 1985 through 1995, an additional 15 years. With the additional follow-up, standardized mortality ratios for lung, tracheal, or bronchial cancer decreased to 1.14 (95% CI: 0.99, 1.30) for battery workers but showed little change for smelter workers at 1.22 (95% CI: 1.00, 1.47). An elevated standardized mortality ratio for stomach cancer (1.53 [95% CI: 1.12, 2.05]) persisted among battery workers, with a lesser elevation among smelter workers (1.33 [95% CI: 0.75, 2.20]). Among other cancers, only thyroid cancer among all workers combined showed a significantly elevated standardized mortality ratio (3.08 [95% CI: 1.33, 6.07]). As with earlier analyses based on this cohort, concomitant exposures to other compounds could not be controlled for, but as these were likely to be most intense among Pb production workers, whose standardized mortality ratios were similar to or

lower than those for battery workers, any bias resulting from such exposure probably was minimal. No data were available to assess the possible role of smoking, diet, or other potential nonoccupational risk factors in the results.

Anttila et al. (1995) linked 20,700 Finnish workers whose blood Pb was monitored during 1973 to 1983 by the Finnish Institute of Occupational Health to the Finnish Cancer Registry. Exposure was subdivided according to highest peak blood Pb measured: low (0 to 0.9 µmol/L [0 to 18.6 µg/dL]), moderate (1.0 to 1.9 µmol/L [20.7 to 39.4 µg/dL]), and high (2.0 to 7.8 µmol/L [41.4 to 161.6 µg/dL]). The total cohort showed no elevation in cancer mortality based on standardized mortality ratio analyses. Among male workers with moderate exposure, however, incidence of total respiratory cancer and lung cancer both were elevated (standardized incidence ratio of 1.4 [95% CI: 1.0, 1.9] for both). Risks of total digestive, stomach, bladder, and nervous-system cancer also were modestly elevated. Risks of mortality for all cancer for both men and women (relative risk of 1.4 [95% CI: 1.1, 1.8]) and lung or tracheal cancer (relative risk of 2.0 [95% CI: 1.2, 3.2]) were even stronger when a person-year analysis was applied to compare workers with moderate Pb exposure to those with low exposure. Risks did not increase in the highest exposure group, although the power of analyses specific for this group were limited by its relatively small size (e.g., lung or tracheal cancer deaths among men in the low-, moderate-, and high-exposure groups numbered 25, 34, and 11, respectively, for the person-year-based analyses).

In summary, occupational exposure to Pb was associated with elevated risks of cancers of the lung and stomach. However, the modest elevation of lung cancer risk seen in most relevant studies is in the range of possible confounding due to smoking or other occupational exposures, particularly arsenic, which precludes the evidence from these studies being seen as conclusive. In particular, one occupational study with the highest lung cancer risk (standardized incidence ratio of 5.1 [95% CI: 2.0, 10.5] with a latency period of 15 years among workers with the highest Pb exposure) (Lundström et al., 1997) has been subsequently shown to be highly confounded by arsenic, and without this study, the combined evidence for a lung cancer elevation across studies is considerably reduced (e.g., the estimated relative risk falls from 1.30 to 1.14). A moderate elevation of stomach cancer is also found in most studies of occupationally-exposed populations with applicable data on this outcome. As with lung cancer, it is possible that other risk factors such as intake of smoked meats or *H. pylori* infection could

have contributed to the observed associations, but the observed elevation (combined risk estimate of 1.33 or 1.34) coupled with the known effect of diet makes it unlikely that the elevation in stomach cancer is entirely due to confounding by diet. Data for other sites such as kidney, brain, and bladder show some indications of an excess, but the results across studies are not consistent and are based on small numbers.

6.7.5.3 Key Studies of the General Population

There are two key general population cohort studies in which Pb exposure was assessed via blood Pb levels (see Annex Table AX6-7.3 for additional details). Jemal et al. (2002) conducted the first biomarker-based general population cohort study of Pb exposure and cancer. The study employed the subsample of 3,592 White U.S. participants in NHANES II (1976 to 1980) who had undergone blood Pb level determinations at time of entry. Deaths among this population were enumerated through 1992 by linkage to the National Death Index (NDI) and Social Security Administration Death Master File. Median blood Pb levels in this population were 12 μg/dL. Adjusted for age, smoking, drinking, region, year, and gender, risk of mortality from any cancer rose across quartiles of blood Pb level, but this trend was not statistically significant. The trend across quartiles was not consistent in gender-specific analyses, although relative risks were elevated for the highest quartile of blood Pb level in both men and women (relative risk 2.0 for men and 1.6 for women). The relative risk for lung cancer based on comparison of subjects with blood Pb levels above or below the median was 1.5 in the combined population, with higher risk observed among women than men. The highest relative risks were observed for cancer of the esophagus (3.7 [95% CI: 0.2, 89]), pancreas (3.6 [95% CI: 0.6, 19.8]), and stomach (2.4 [95% CI: 0.3, 19.1]); no elevations were noted for cancers of other sites. Total cancer mortality was also addressed through a spline regression (Figure 6-13). The mortality curves were visually suggestive of an upward trend at low blood Pb levels (<20 μg/dL), but no statistically significant dose-response pattern was present except for analyses restricted to women.

The lack of statistically significant results reflects the small number of deaths during follow-up, which limited the study's power; of the nine major sites examined, the number of deaths ranged between 5 and 16 for all sites except the lung. Further, only 4 and 16 deaths

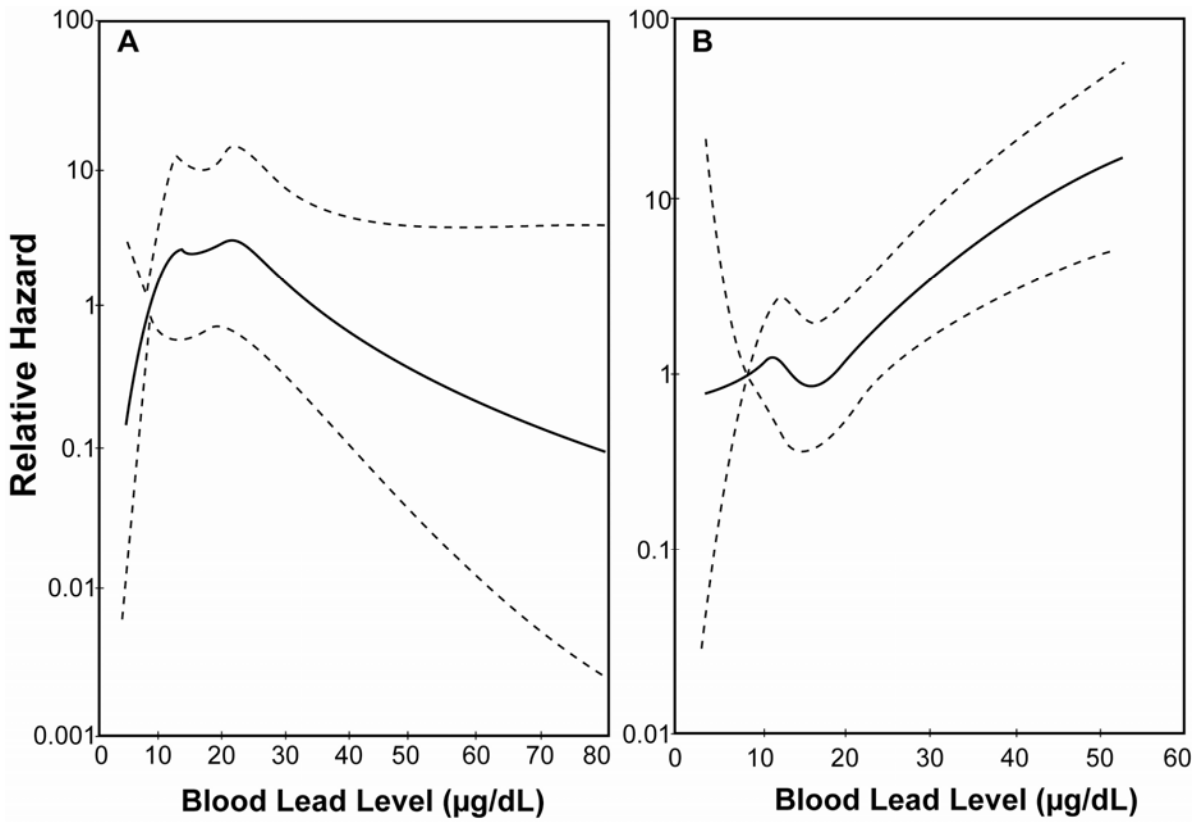

Figure 6-13. Five-knot cubic spline regression models of total cancer mortality and blood lead level by gender, based on analyses of the NHANES II cohort. Relative risk of all cancer mortality for different blood lead levels compared with referent blood lead level of 8 µg/dL (the 12.4th percentile) among White men (A) and White women (B) in the United States (NHANES II). The solid line shows the fitted 5-knot spline relationship; the dashed lines are the point wise upper and lower 95% confidence limits.

Source: Jemal et al. (2002).

occurred among men and women, respectively, with blood Pb levels <9.8 µg/dL, precluding assessment of potential effects within that range. Detailed exposure-response analyses were restricted to all cancers combined, although potential effects could have been strongly target-organ specific. In addition, the use of quartile cut points based on the distribution of Pb concentrations estimated for the total U.S. population resulted in relatively small numbers in the referent group (lowest exposure quartile) for males and in the high-exposure quartile for females. Use of a biomarker provided an objective measure of Pb exposure. Nevertheless, reliance on a

single blood Pb measurement produces less reliable estimates than would be obtained through multiple measurements and precludes addressing temporal changes in Pb exposure over the follow-up period. Lack of control for exposure to occupational carcinogens other than Pb and potential residual confounding by duration and intensity of tobacco smoking also could have biased the results, especially for men.

Lustberg and Silbergeld (2002) carried out another biomarker-based general population study based on the same NHANES II mortality cohort used by Jemal et al. (2002). This study did not exclude non-Whites, however, and employed more extensive adjustment for potential confounding factors than the Jemal et al. (2002) analyses (i.e., education, body mass index, and exercise were included in the regression models, although alcohol intake was not). In addition, persons with blood Pb levels ≥30 μg/dL were excluded in order to restrict comparisons to levels below the OSHA standard for Pb exposure. Persons with levels below 10 μg/dL served as the referent group. Survival analyses adjusted for potential confounders found a relative risk for cancer mortality of 1.5 (95% CI: 0.9, 2.5) for those with blood Pb levels of 10 to 19 μg/dL and 1.7 (95% CI: 1.0, 2.8) for those with levels of 20 to 29 μg/dL. Separate analyses of lung-cancer and non-lung-cancer deaths yielded estimates of increased risk for moderate- or high-exposure groups, compared with the referent population, both for lung cancer and non-lung cancer. However, none of the estimates reached the $p < 0.05$ level of statistical significance, and the results for non-lung cancers showed no evidence of an exposure-response relationship.

As with Jemal et al. (2002), the use of a biomarker for exposure and the prospective design of the study are strengths. Its attempts to control for potential confounders were more extensive, and its choice of cut points for the referent category yielded more males in the referent group, although that group still included less than 20% of the study population. However, it is notable that blood Pb levels rose significantly with smoking level. The models included terms for former smoking, current light smoking, and current heavy smoking (>1 pack per day). Still, some degree of residual confounding due to smoking might have remained, which could have contributed to the estimated risk of lung cancer for the highest exposure category (relative risk of 2.2 [95% CI: 0.8, 6.1]). Such residual confounding would have had less effect on the results for non-lung cancer. As noted regarding the other NHANES-based study, however, mortality due to cancers of other sites was too uncommon to allow for reliable site-specific comparisons. In the Lustberg and Silbergeld analysis, all cause and cardiovascular mortality increased monotonically

with blood Pb level, which might indicate residual confounding from SES or smoking affecting both heart disease and cancer.

6.7.5.4 Other Lead Studies

There are a variety of other epidemiologic studies of Pb exposure, which are less important than the key studies above but which offer some information. Studies reviewed in this section are summarized in Annex Table AX6-7.4. The weaknesses in these studies largely stem from potential confounding by other metals in the cohort studies and likely misclassification of Pb exposure in the case-control studies.

Some studies have examined the potential link between parental Pb exposure and childhood cancer. These are briefly described in Section 5.6.2.1, but are not further detailed here. Lack of direct measures of child exposure, the fact that many of the same interpretational problems (e.g., potential coexposures) noted for occupational studies as a whole, and low statistical power due to the rarity of the outcome under study render the available evidence less relevant than that from direct study of exposed occupational and general populations.

6.7.6 Confounding of Occupational Lead Studies Due to Other Occupational Exposures: Arsenic, Cadmium

A number of studies of Pb workers come from smelters, where exposures to other metals are common. Of particular concern are other lung carcinogens, not only especially arsenic (workers exposed to high levels of arsenic historically have had a lung cancer relative risk of 3 to 4, see Steenland et al. 1996), but also Cd. Glass workers are also of limited use for inference about Pb effects, as they are also typically exposed to Cd, arsenic, chromium, and nickel, all of which are lung carcinogens (e.g., see Wingren and Axelson, 1993).

In some smelters, measurements have been taken which indicate clearly that exposures to these other carcinogens was minimal and the main suspect is Pb (e.g., Steenland et al., 1992). In others, however, one is unable to disentangle the effects of arsenic and Pb (Ades and Kazantis, 1988, Lundström et al., 1997). As a result, these studies cannot yield strong evidence regarding the possible relation between lung cancer and Pb specifically. The study by Lundström et al., 1997 is particularly important in this regard, because it had a high relative risk of 2.8 (95% CI: 2.0, 3.8), and had an important effect in raising the overall result when included in meta-analyses

(e.g., Steenland and Boffetta [2000], where exclusion of the Lundström et al. study lowered the estimated combined lung cancer relative risk from 1.30 to 1.14). A subsequent publication by Englyst et al. (2001) indicated that the smelter workers studied by Lundström et al. (1997) likely had significant exposure to arsenic, and the authors concluded that it was impossible to separate the effects of Pb and arsenic.

6.7.7 Confounding of Lead Studies: Smoking and Other Factors

The most informative studies of Pb carcinogenicity are those comparing highly exposed workers to general populations. In these comparisons one must consider typical differences between worker populations and the general populations, in particular differences due to smoking and diet. Smoking can be a major confounder for lung cancer, while diet or SES can be a confounder, albeit weaker, for stomach cancer.

Regarding smoking, it has been shown both theoretically and empirically that confounding due to smoking differences between workers and the general population will typically account for an observed relative risk of ~1.1 to 1.2, with a possible maximum of about 1.4 (Axelson and Steenland, 1988; Siemiatycki et al., 1988). Furthermore, most occupational cohort studies are retrospective and have little information on smoking, making it impossible to control directly for potential confounding by this strong risk factor. As noted above, the lung cancer relative risk in the meta-analysis of Steenland and Boffetta (2000), after excluding the Lundström et al. study, was 1.14 (95% CI: 1.04, 1.73), based on seven occupational cohort studies, six of which used a non-worker external referent population, and none of which controlled for smoking as a confounder. This relatively small excess relative risk could plausibly be due to confounding by smoking. Unfortunately the occupational cohort studies were usually not followed by nested-case control studies of lung cancer, which could have controlled for smoking and, furthermore, they usually did not involve internal exposure-response analyses, wherein confounding by smoking is usually minimal. An exception was the lung cancer case-control study conducted by Anttila et al. (1995) within a large cohort of Finnish workers with known blood Pb levels. In this case-control study smoking-adjusted lung cancer odds ratios were increased among workers with higher estimated cumulative blood Pb or higher peak blood Pb exposure compared to workers with the lowest exposure, and the authors noted that smoking actually appeared to be a “weak negative confounder” for the high peak blood Pb

group. Also, in one large population-based case-control study with extensive information on other cancer risk factors, there remained an elevated odds ratio for lung cancer with substantial Pb exposure after controlling for smoking (Siemiatycki et al., 1991). Hence, there is some evidence that confounding by smoking does not likely explain the modest excess lung cancer risk seen in many studies.

Diet high in salt or smoked meats, *Helicobacter pylori* infection, and SES are possible confounders for stomach cancer. Those of highest SES compared to those of lower SES have been shown to have a relative risk of about 3.0 (Tomatis, 1990). None of the occupational cohort studies, in which again stomach cancer in workers was compared to the general population, controlled for these potential confounders. However, these potential confounding factors are much less powerful risk factors in respect to stomach cancer than smoking is with respect to lung cancer, and hence are unlikely to account for relative risks higher than perhaps 1.1 or at most 1.2. Given that the occupational cohort studies had a combined relative risk of 1.34 (95% CI: 1.14, 1.57) in the meta-analysis of Steenland et al. (2002) and 1.33 (95% CI: 1.18, 1.49) in that of Fu and Boffetta (1995), it seems unlikely that confounding by these factors can fully account for the excess stomach cancer risk observed in the occupational studies.

6.7.8 Summary of Epidemiologic Evidence for the Genotoxic and Carcinogenic Effects of Lead

The availability of studies of cancer in Pb-exposed populations was limited at the time of the 1986 Lead AQCD. The number and range of studies have since notably expanded, including extended follow-ups of previous cohorts, new cohort and case-control studies, and analyses addressing not only cancer but genotoxicity. The newly available epidemiologic data greatly enhance the knowledge base regarding Pb carcinogenicity, with key findings and conclusions emerging as follows.

- Studies of genotoxicity consistently link Pb-exposed populations with DNA damage and micronuclei formation, although less consistently with chromosomal aberrations, a more established indicator of cancer risk.

- The epidemiologic data reviewed above from key high Pb exposure occupational studies suggest a relationship between Pb exposure and cancers of the lung and the stomach, as supported by two meta-analyses. Clear conclusions are limited by potential confounders, e.g., other occupational exposures (arsenic, Cd), smoking, and dietary habits.

- Two general population cohort studies (NHANES II) have been conducted. Low Pb exposure was assessed via blood levels. These studies show internal dose-response trends but suffer at times from small numbers for site-specific analyses or lack of site-specific analyses altogether, and from possible residual confounding by smoking and SES.

- Overall, the above findings provide only very limited evidence suggestive of Pb exposure associations with carcinogenic or genotoxic effects in humans. On the other hand, animal studies (see Chapter 5) provide reproducible results in several laboratories and multiple rat strains (with some evidence of multiple tumor sites), along with short-term studies indicating that Pb affects gene expression. However, although the animal studies clearly demonstrate Pb-related carcinogenic effects in response to dietary and subcutaneous exposures to several soluble Pb salts, they may be of limited relevance here because human exposures of most concern here are to inhaled Pb oxides.

- Nevertheless, the most recent IARC (2005) review concluded that inorganic Pb compounds were probable human carcinogens (Group IIA), based on limited evidence in humans and sufficient evidence in animals. This is consistent with the U.S. National Toxicology Program's Carcinogen Review Committee Report, which recommends that Pb and Pb compounds be considered as "reasonably anticipated humans carcinogens." Similarly, although the human evidence is inadequate according to EPA's Guidelines for Carcinogen Risk Assessment (U.S. Environmental Protection Agency, 2005), Pb is likely classifiable under those guidelines as a probable human carcinogen based on the available animal data.

6.8 EFFECTS OF LEAD ON THE IMMUNE SYSTEM

6.8.1 Summary of Key Findings of the Effects of Lead on the Immune System from the 1986 Lead AQCD

The 1986 Lead AQCD concluded that studies conducted in laboratory animal models provided evidence for immunosuppressive effects of Pb; however, evidence for such effects in humans was lacking. Since then, the epidemiological study of immunological effects of Pb has progressed considerably. The currently available epidemiologic and clinical observations are consistent with the greater body of evidence derived from studies in experimental animals indicating that Pb can suppress cellular and humor immunity and decrease host resistance to infection agents and tumor cells (see Section 5.9). Findings from the epidemiologic studies suggest that Pb exposure (as reflected in blood Pb concentration) may be associated with effects on cellular and humoral immunity. These effects include changes in serum immunoglobulin levels (e.g., elevated serum IgE); perturbation of peripheral lymphocyte phenotype profiles, including decreases in peripheral blood T-cell abundance and changes in T-cell:B-cell abundance

ratios; suppression of lymphocyte activation; and suppression of neutrophil chemotaxis and phagocytosis.

Available studies of associations between Pb exposure and immunological outcomes are summarized in Annex Tables AX6-8.1 and AX6-8.2. In general, while the studies provide support for associations between Pb exposure and immunological outcomes, the studies have numerous limitations that complicate the assessment of the strength of reported associations and potential causation. Furthermore, the health consequences of outcomes that have been associated with Pb exposure are uncertain. All studies have been cross-sectional in design and most included relatively small cohorts. The studies implemented varying degrees of quantitative analysis of potential covariables and confounders. In most studies, a detailed analysis of covariables and confounding was lacking, and many of the reports offered no analysis of covariables or confounding. Covariables that were considered (but not consistently) in multivariate analyses or controlled by stratification included age, sex, race, smoking habits, alcohol consumption, and illness and/or medications that might affect the immune system. Studies that offer the strongest designs are discussed in greater detail below.

6.8.2 Host Resistance, Hypersensitivity, and Autoimmunity

Associations between Pb exposure and host resistance have not been rigorously examined in humans. Two analyses of illness surveys in children (Rabinowitz et al., 1990) and Pb workers (Ewers et al., 1982) have been reported. Both studies relied on personal surveys for assessment of illness and neither study considered covariates or confounders in the analyses. In the Rabinowitz et al. (1990) study, the highest relative risks (blood Pb concentration ≥10 μg/dL compared to <10 μg/dL) were: other respiratory tract illnesses, 1.5 (95% CI: 1, 2.3); severe ear infections, 1.2 (95% CI: 1, 1.4); illnesses other than cold or influenza, 1.3 (95% CI: 1.0, 1.5). Ewers et al. (1982) reported mean frequency of self-reported colds and influenza per year of employment in Pb workers (blood Pb range 21-85 μg/dL) compared to a reference group (range 6-21 μg/dL). Mean frequency of 2 to 4 illnesses per year was higher among the Pb workers 28.8% versus 16.1%); however, a statistical analysis of the data was not reported. Collectively, these studies do not provide convincing evidence for a strong association between Pb exposure and altered disease resistance in humans.

Two studies have also been reported that have examined possible associations between Pb exposure (e.g., blood Pb concentration) and asthma. In the Rabinowitz et al. (1990) study described above, the relative risk of asthma (blood Pb <5 μg/dL compared to ≥5 or ≥10 μg/dL) were not significant in Caucasian or African-American cohorts. In the Caucasian cohort, the hazard ratios were 1.4 (95% CI: 0.7, 2.9) for ≥5 μg/dL and 1.1 (95% CI: 0.2, 8.4) for ≥10 μg/dL. In the African-American cohort, the corresponding hazard ratios were 1.0 (95% CI: 0.8, 1.3) for ≥5 μg/dL and 0.9 (95% CI: 0.5, 1.4) for ≥10 μg/dL. Hazard ratios for asthma incidence in African-Americans compared to Caucasians (<5 μg/dL) were 1.6 (95% CI: 1.4, 2.0) for <5 μg/dL, 1.4 (95% CI: 1.2, 1.6) for ≥5 μg/dL, and 2.1 (95% CI: 1.2, 3.6) for ≥10 μg/dL. Thus, while there appeared to be an elevated risk of asthma in the African-American cohort, relative to the Caucasian cohort, a significant effect of blood Pb level on risk in African-Americans or Caucasians was not evident in this study. Covariates included in the analysis were average annual income, birth weight and gender. Similar results were obtained when a more stringent definition of asthma was applied to the subjects. Collectively, these studies do not provide convincing evidence for a strong association between Pb exposure and asthma in children.

6.8.3 Humoral Immunity

A characteristic immunological response to Pb exposure in animals is an increase in production of IgE, immunoglobulin that has been associated with allergy and allergic airway disease (see Section 5.9.3.2). Although epidemiologic literature is not conclusive regarding the dose-response relationships for Pb effects on immunoglobulin production in humans, studies in children have consistently found significant associations between increasing blood Pb level and increasing serum IgE levels (Karmaus et al., 2005; Lutz et al., 1999; Sun et al., 2003) (Table 6-5). These effects were evident at blood Pb values <10 μg/dL. Increasing serum IgE levels also have been observed with increasing blood Pb concentration (blood Pb ≥30 μg/dL) in association with occupational exposures to Pb (Heo et al., 2004). Outcomes for other immunoglobulin indices in adults have been less consistent (Pinkerton et al., 1998; Sarasua et al., 2000).

Possible associations between Pb exposure and biomarkers of humoral immunity in children have been examined in several cross-sectional studies (Annesi-Maesano et al., 2003;

Table 6-5. Summary of Results of Selected Studies of Associations Between Lead Exposure and Serum Immunoglobulin Levels

Study	Subjects	n[a]	Blood Lead (µg/dL)		IgA	IgE	IgG	IgM
			Mean (SD)	Range				
Children								
Annesi-Maesano et al. (2003)	Neonates	374	67 (48)[b]	NR	NR	+[c]	NR	NR
Karmaus et al. (2005)	Children, 7–10 yr	331	3	1–5[e]	o	+	o	o
Lutz et al. (1999)	Children, 9 mo–6 yr	270	NR	1–45	NR	+	NR	NR
Sarasua et al. (2000)	Children, 6–35 mo	372	7	~2–16[d]	+	NR	+	+
Sun et al. (2003)	Children, 3–6 yr	73	NR	~3–40	NR	+	−	−
Adults								
Heo et al. (2004)	Batter manufacture workers	606	~22 (~10)[e]	NR	o	+	o	o
Pinkerton et al. (1998)	Smelter workers	229	39[f]	<2–55	o	NR	−	o
Sarasua et al. (2000)	General population	433	4.3	~1–10[d]	o	NR	o	o

−, decrease; +, increase; o, no effect; NR, not reported; Ig, serum immunoglobulin level.

[a] Total number of subjects (including reference group)
[b] Infants cord blood (maternal blood lead mean was 96 µg/dL [SD 58])
[c] In association with increasing neonatal hair lead
[d] 5th–95th percentile range
[e] Mean of age-group means and SDs
[f] Median

Karmaus et al., 2005; Lutz et al., 1999; Reigart and Graher, 1976; Sarasua et al., 2000; Sun et al., 2003; Wagnerova et al., 1986). Four studies warrant particular attention because they examined a relatively low range of blood Pb concentrations and applied multivariate analyses to the data in attempts to control for possible covariables (Karmaus et al., 2005; Lutz et al., 1999; Sarasua et al., 2000; Sun et al., 2003). Three studies found significant associations between increasing blood Pb concentration and serum IgE levels (Karmaus et al., 2005; Lutz et al., 1999; Sun et al., 2003). The reported percent increase in serum IgE levels measured in these studies ranged from ~50 to 400%. The Lutz et al. (1999) study measured serum IgE and IgG (against Rubella) in 270 children (age range 9 months to 2 years; blood Pb range 1 to 45 μg/dL). The observed blood Pb-age-IgE relationship is shown in Figure 6-14. The highest IgE levels (mean 211 IU/mL [SD 441], n = 17) were observed in children who had blood Pb concentrations in the range of 15 to 19 μg/dL; by comparison, mean IgE levels were 52 IU/mL (SD 166) for subjects who had blood Pb concentrations <10 μg/dL (n = 174). The Karmaus et al. (2005) study measured serum IgA, IgE, IgG, and IgM levels in 331 children (age range 7-10 years). Blood Pb levels were lower in this study than in the Lutz et al. (1999) study (1 to 5 μg/dL). A multivariate linear regression analysis revealed a significant association between blood Pb ($p < 0.05$) and serum IgE (but not IgA, IgG, or IgM). The change in serum IgE level may appear not to be monotonic with increasing blood Pb concentration (Figure 6-15). However, the two lowest means are not significantly different so that apparent non-monotonicity of the effect/exposure relationship does not have statistical support. The highest IgE levels (adjusted mean 59 IU/L) were observed in the children who had blood Pb concentrations ranging from 2.8 to 3.4 μg/dL (n = 86) and >3.4 μg/dL (n = 82). Sun et al. (2003) measured serum IgE, IgG, and IgM levels in children, ages 3 to 6 years (blood Pb range 2.6–44 μg/dL, n = 73). A nonparametric comparison of immunoglobulin levels between low (<10 μg/dL) and high (≥10 μg/dL) blood Pb strata revealed significantly higher IgE levels and significantly lower IgG and IgM levels in the high blood Pb stratum.

The study by Annesi-Maesano et al. (2003) provides futher suggestive evidence for an association between Pb exposure and increasing IgE levels. The study included 374 mother-infant pairs who had relatively high mean blood Pb levels (maternal mean 96 μg/dL [SD 58]; infant cord 67 μg/dL [SD 48]). Serum IgE level was significantly associated with increasing infant hair Pb ($p < 0.001$), but not with cord blood Pb or placental Pb level. The association

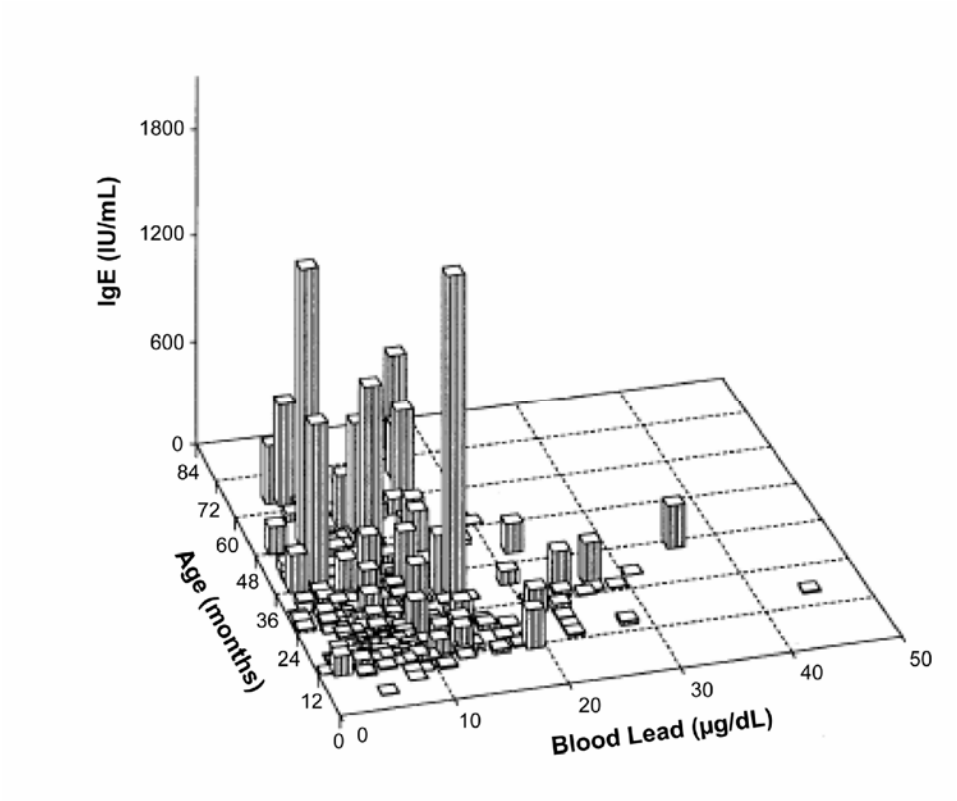

Figure 6-14. Relationship between blood lead concentration, age, and serum IgE level in children. Spearmen partial correlation between blood lead and serum IgE is 0.22 (p = 0.0004, n = 221).

Source: Lutz et al. (1999).

between IgE and hair Pb levels was evident in a subset of mother-infant pairs, in which mothers were classified as nonallergenic, and was unrelated to maternal smoking (i.e., urinary cotinine). The ATSDR Multisite Lead and Cadmium Exposure Study (ATSDR, 1995) is one of the largeststudies to assess humoral immune status in association with Pb exposures; however, it did not include an assessment of IgE. The study included a cross-sectional analysis of serum IgA, IgG, and IgM levels in 1,561 subjects (age range 6 months to 75 years) who resided in areas impacted by Pb mining and/or smelting operations and in 480 demographically-matched controls (Sarasua et al., 2000). A multivariate linear regression analysis of immunoglobulin levels and blood Pb concentration (exposed and control groups combined) revealed associations between

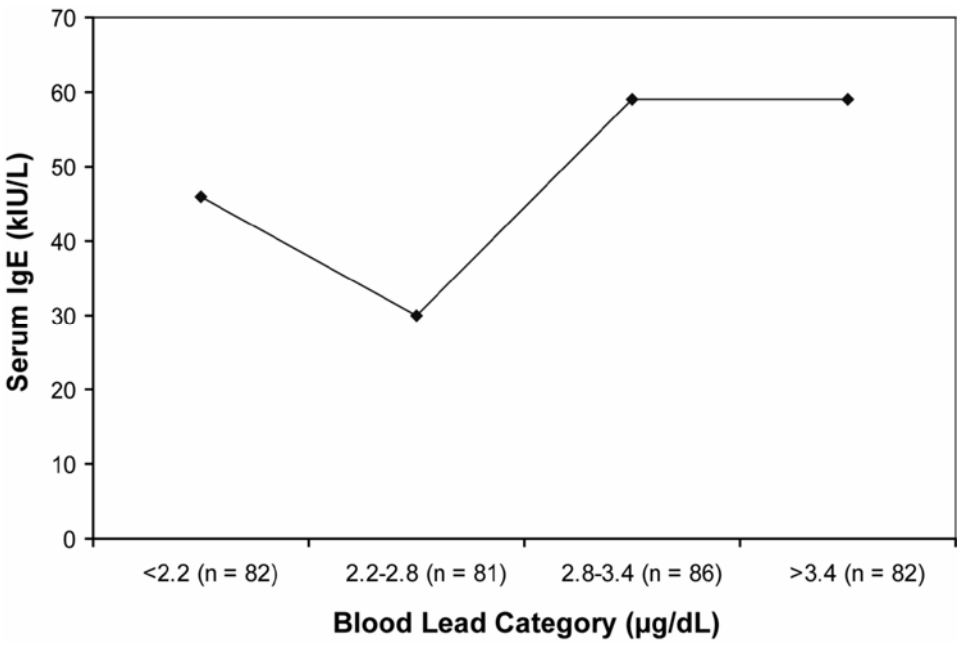

Figure 6-15. Relationship between blood lead concentration and serum IgE level in children. Mean serum IgE levels (standard deviations not reported) are adjusted for age, number of infections in the previous 12 months, exposure to passive smoke in the previous 12 months, and serum lipids (sum of cholesterol and triglycerides). Means of serum IgE levels in blood lead categories were significantly different (F-test p = 0.03).

Source: Karmaus et al. (2005).

increasing blood Pb and increasing serum IgA, IgG, and IgM levels in subjects 6 to 35 months of age (blood Pb 5th–95th percentile range 1.7–16 µg/dL, Figure 6-16).

Possible associations between Pb exposure and biomarkers of humoral immunity also have been examined in several cross-sectional studies of Pb workers (Alomran and Shleamoon, 1988; Anetor and Adeniyi, 1998; Ayatollahi, 2002; Coscia et al., 1987; Ewers et al., 1982;

Heo et al., 2004; Kimber et al., 1986; Pinkerton et al., 1998; Ündeğer et al., 1996). Outcomes from these studies, with respect to humoral immune parameters, measured as serum and/or salivary immunoglobulin levels, are mixed. Some studies finding positive associations with blood Pb (Heo et al., 2004), negative associations (Anetor and Adeniyi, 1998; Ewers et al., 1982; Pinkerton et al., 1998), or no (or mixed) effects (Alomran and Shleamoon, 1988; Kimber et al., 1986; Queiroz et al., 1994b; Sarasua et al., 2000; Ündeğer et al., 1996). Based on study

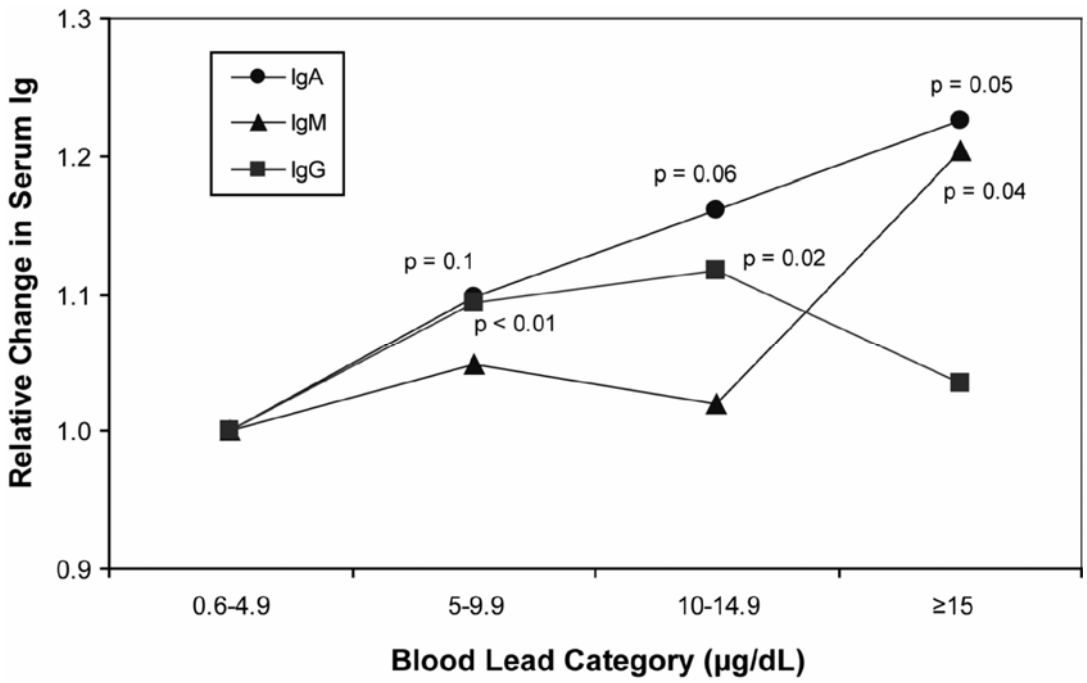

Figure 6-16. Relationship between blood lead concentration and serum immunoglobulin (Ig) levels in children. Shown are relative changes in serum Ig levels, adjusted for age, sex, and exposure location. P-values reflect comparison to <5 µg/dL blood lead category mean (<5 µg/dL, n = 165; 5-9.9 µg/dL, n = 136; 10–14.9 µg/dL, n = 47; ≥15 µg/dL, n = 24).

Source: Sarasua et al. (2000).

design considerations (e.g., cohort criteria, size, treatments of covariates), three studies warrant particular attention (Heo et al., 2004; Pinkerton et al., 1998; Sarasua et al., 2000). Of these, only Heo et al. (2004) assessed serum IgE levels consistent with outcomes reported in children, increasing blood Pb concentration was significantly associated with increasing serum IgE levels (Figure 6-17). The study measured serum IgE, IL-4 and IFNγ in 606 battery manufacture workers. Serum IgE levels were significantly higher in the blood Pb stratum (≥30 µg/dL) compared to lower strata (<10 or 10–29 µg/dL) for the age strata 30–39 years, ≥40 years, and for all ages combined.

Although the Pinkerton et al. (1998) study did not assess IgE outcomes, it offers the strongest study design of the three for assessment of other immunoglobulin classes. Even though it is a relatively small cross-sectional study, it considered immune illnesses and immune

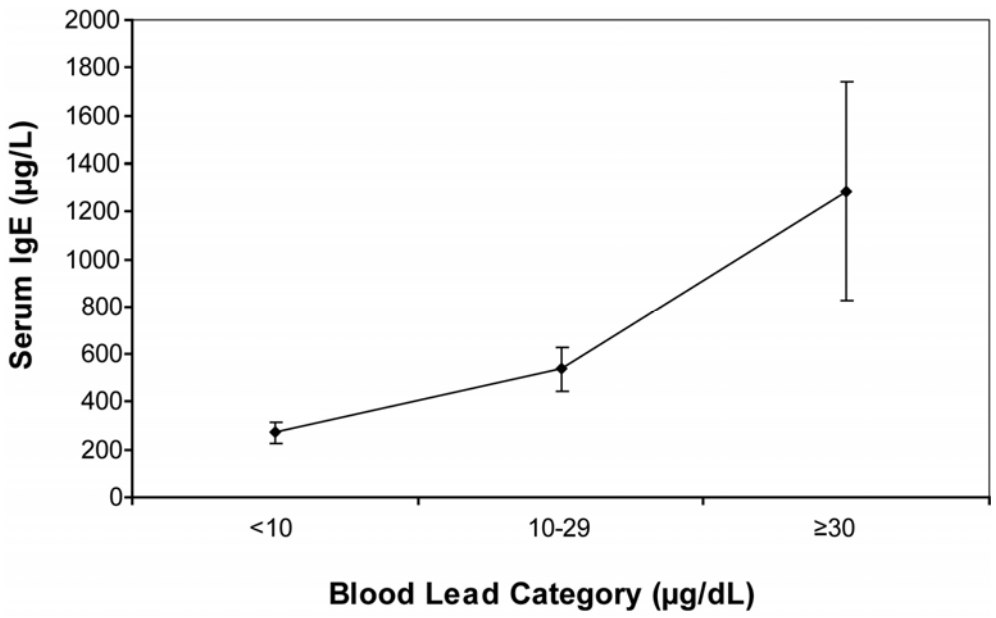

Figure 6-17. Relationship between blood lead concentration and serum IgE level in lead workers. Mean serum IgE levels in high blood lead category were significantly higher for all ages (shown), and within age categories ≥40 years and 30-39 years, but not within age category <30 years.

Source: Heo et al. (2004).

suppressant drugs in the construction of the cohorts and examined a relatively large number of potential covariates in the data analysis. Serum immunoglobulin levels were measured in male smelter (n = 145) workers and hardware workers (n = 84). Excluded (by blind evaluation) from the study cohorts were individuals who had "serious" illnesses of the immune system, who were taking immune suppressant drugs, or who had chemical exposures (other than to Pb) that might affect immune function. Median blood Pb concentrations were 39 µg/dL (range 15–55 µg/dL) in the Pb workers and <2 µg/dL (range <2–12 µg/dL) in the reference group. Covariate-adjusted (logistic regression) geometric mean serum IgA, IgG, and IgM, and salivary IgA levels in the Pb workers were not significantly different from the reference group; however, the adjusted regression coefficient for serum IgG and time-integrated (but not current) blood Pb level was negative and significant.

The Sarasua et al. (2000) study, described above for its assessment of children, also included a cross-sectional analysis of serum IgA, IgG, and IgM levels in adults (age 16–75 years,

n = 433; blood Pb 5th–9th percentile range 1 to 10 μg/dL) and found no significant associations between blood Pb and serum immunoglobulin levels (serum IgE outcomes were not assessed).

Also germane to the evidence for effects of Pb on humoral immunity in humans are the results of a clinical study in which serum immunoglobulin levels were repeatedly measured in a Pb smelter worker who underwent CaEDTA chelation therapy three times per week for a period of 10 weeks (Sata et al., 1998). Serum IgA, IgG, and IgM were significantly higher when assessed 24 h after each CaEDTA treatment compared to assessments made prior to treatment. Furthermore, serum IgG levels were significantly negatively correlated with blood Pb level during the treatment period. Before-treatment and after-treatment blood Pb concentration means were 45.1 μg/dL (SD 16.0) and 31.0 μg/dL (SD 9.8), respectively.

6.8.4 Cell-Mediated Immunity

Studies conducted in animals and in vitro experimental models indicate that Pb preferentially targets macrophages and T lymphocytes (see Section 5.9.4). However, the prominent effects are largely on immune system function, rather than overt cytotoxicity to lymphoid tissues. Lead suppresses Th1-dependent responses (e.g., delayed type hypersensitivity) and production of Th1 cytokines; and stimulates macrophages into a hyperinflammatory state. These types of functional changes have not been rigorously evaluated in human epidemiological studies, which have relied, for the most part, on changes in lymphocyte abundance as the main outcomes for assessing status of cellular immune systems. Lead-induced functional changes in immune responses may not be reflected in changes in lymphocyte abundance and, correspondingly, specific functional changes may not be readily discerned from observed changes in lymphocyte abundance. Studies of children have found significant associations between increasing blood Pb level and decreases in T-cell abundance, with corresponding increases in B-cell abundance (Karmaus et al., 2005; Sarasua et al., 2000; Zhao et al., 2004). These effects have been observed in children whose blood Pb concentrations were <10 μg/dL (Karmaus et al., 2005; Sarasua et al., 2000), although not all studies (e.g., Lutz et al., 1999) have found such associations at higher blood Pb levels (e.g., 10–45 μg/dL). Studies of occupational Pb exposures have also found associations between increasing blood Pb levels and changes (increases or decreases) in T-cell abundance (Fischbein et al., 1993; Pinkerton et al., 1998; Sata et al., 1997). Effects were observed in association with blood Pb concentrations

<25 μg/dL (Fischbein et al., 1993) and in populations whose blood Pb levels ranged from ~7 to 55 μg/dL (Pinkerton et al., 1998; Sata et al., 1997). Outcomes from these studies are qualitatively summarized in Table 6-6 and are discussed in greater detail below.

Several cross-sectional studies have examined possible associations between Pb exposure and biomarkers of cellular immunity in children (Karmaus et al., 2005; Lutz et al., 1999; Sarasua et al., 2000; Zhao et al., 2004). Three studies (Karmaus et al., 2005; Sarasua et al., 2000; Zhao et al., 2004) found significant associations between increasing Pb exposure and decreases in T-cell abundance (Table 6-6). The largest study (Sarasua et al., 2000) examined abundance of total lymphocytes, T-cells ($CD3^+$), B-cells ($CD20^+$), NK cells, and $CD4^+$ and $CD8^+$ T-cell phenotypes in infants, children, and adolescents. Associations between increasing blood Pb concentration and increasing B-cell abundance (% and number), and decreasing T-cell abundance (%) were found for children 6–35 months of age (n = 312), after adjustment for age, sex, and study site (of four mining/smelting sites). Comparison of adjusted means for outcomes across blood Pb strata revealed that the differences were significant for the ≥15 μg/dL stratum only, compared to the <5 μg/dL stratum. The Karmaus et al. (2005) study examined children in the age range of 7 to 10 years (n = 331) with blood Pb levels <5 μg/dL. In addition to age and sex, regression models relating outcomes to blood Pb concentration included exposure to environmental tobacco smoke and infections in the previous year as covariates. Similar to the Sarasua et al. (2000) study, Karmaus et al.(2005) found significant associations between blood Pb and decreased T-cell abundance ($CD3^+$, $CD3^+CD8^+$) and increased B-cell ($CD19^+$) abundance (for the blood Pb quartile 2.2 to 2.8 μg/dL) (Figure 6-18). Zhao et al. (2004) examined lymphocyte phenotype abundance in children in the age range 3 to 6 years (n = 73) and found significantly lower % abundance of T-cell phenotypes $CD3^+CD4^+$, $CD4^+CD8^+$ and significantly higher abundance of $D3^+CD8^+$ cells in children whose blood Pb concentrations were ≥10 μg/dL compared to <10 μg/dL. Lutz et al. (1999) found no significant associations between blood Pb concentration and age-adjusted T-cell ($CD3^+$) or B-cell (CD19+) abundance or abundance of various other lymphocyte phenotypes (i.e., $CD2^+$, $CD25^+$, $CD28^+$, $CD71^+$) in children whose blood Pb concentrations were 10 to 14, 15 to 19, or 20 to 45 μg/dL compared to <10 μg/dL.

A larger set of studies have evaluated potential associations between Pb exposure and biomarkers of cellular immunity in adults (Basaran and Ündeğer, 2000; Cohen et al., 1989; Coscia et al., 1987; Fischbein et al., 1993; Kuo et al., 2001; Mishra et al., 2003; Pinkerton et al.,

Table 6-6. Summary of Results of Selected Studies of Associations Between Lead Exposure and Serum Lymphocyte Abundances

Study	Subjects	n[a]	Blood Lead (μg/dL)		T[b]	T_H[c]	T_C[d]	T_{HC}[e]	T_M[f]	NK[g]	B[h]
			Mean (SD)	Range							
Children											
Karmaus et al. (2005)	Children, 7–10 yr	331	3	1–5[i]	−	o	−	NR	o	o	−
Lutz et al. (1999)	Children, 9 mo–6 yr	270	NR	1–45	o	NR	NR	NR	NR	NR	o
Sarasua et al. (2000)	Children, 6–35 mo	372	7	~2–16[i]	−	o	o	NR	NR	o	+
Zhao et al. (2004)	Children, 3–6 yr	73	NR	~3–40	o	−	+	−	NR	NR	o
Adults											
Fischbein et al. (1993)	Firearms instructors	87	31 (4)[j]	NR	−	−	o	NR	NR	o	+
Pinkerton et al. (1998)	Smelter workers	229	39[k]	<2–55	o	o	o	o	+	o	+
Sarasua et al. (2000)	General population	433	4.3	~1–10[i]	o	o	o	o	NR	o	o
Sata et al. (1997)	Lead stearate workers	99	19	7–50	o	o	+	NR	−	NR	o

−, decrease; +, increase; o, no effect; NR, not reported.

[a] Total number of subjects (including reference group)
[b] T-cells ($CD3^+$)
[c] T-helper cells ($CD4^+$)
[d] Cytotoxic T-cells ($CD8^+$)
[e] $CD4^+CD8^+$
[f] T-memory cells ($CD45RO^+$, $CD45RA^+$)
[g] Natural killer cells (e.g., $CD16^+$, $CD56^+$)
[h] B-cells (e.g., $CD19^+$, $CD20^+$)
[i] 5th–95th percentile range
[j] High exposure group
[k] Median

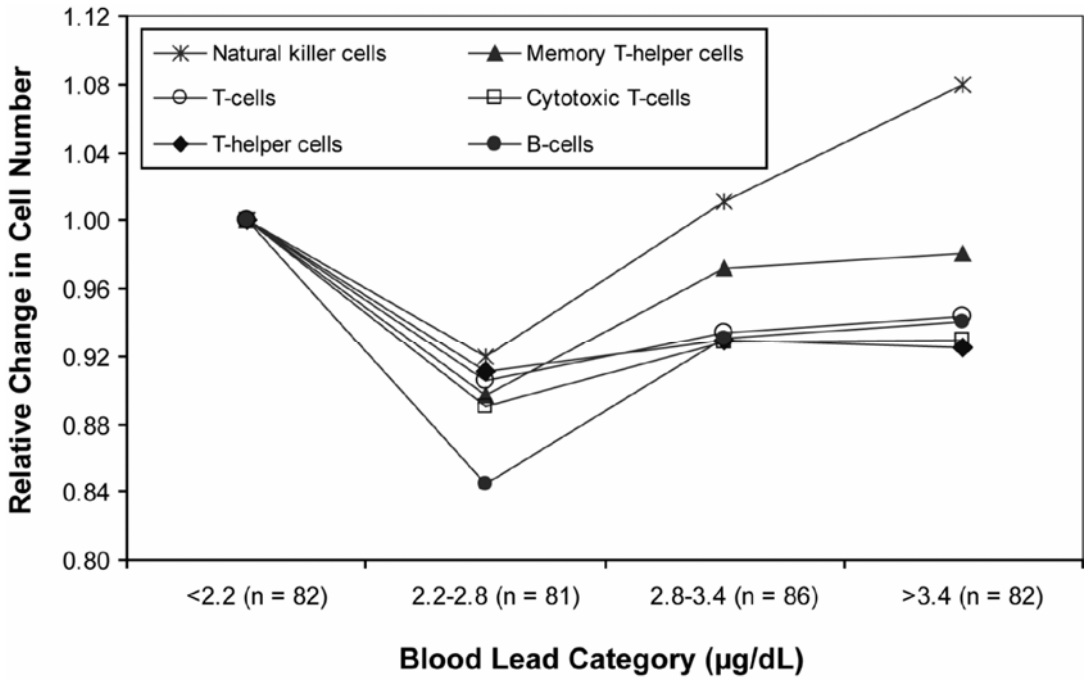

Figure 6-18. Relationship between blood lead concentration and T- and B-cell abundances in children. Shown are relative changes in covariate-adjusted absolute cell numbers (cells/µL) compared to the lowest blood lead group; adjusted for age, number of infections in the previous 12 months, exposure to passive smoke in the previous 12 months, and serum lipids (sum of cholesterol and triglycerides). Abundances for T-cells, cytotoxic T-cells, and B-cells in the 2.2-2.8 µg/dL group were significantly different ($p \leq 0.05$) from the <2.2 µg/dL group. Receptor phenotypes assayed were: T-cells, CD3+; T-helper cells, CD3+CD4+; cytotoxic T-cells, CD3+CD8+; memory T-helper cells, CD4+CD45RO+; natural killer cells, CD16+CD56+; B-cells, CD3+CD5+CD19+.

Source: Karmaus et al. (2005).

1998; Sarasua et al., 2000; Sata et al., 1998, 1997; Yücesoy et al., 1997b; Ündeğer et al., 1996). Four studies warrant particular attention because they implemented relatively stronger study designs (i.e., cohort criteria, size, treatment of covariates): Fischbein et al., 1993; Pinkerton et al., 1998; Sarasua et al., 2000; Sata et al., 1998). With one exception (Sarasua et al., 2000), all were studies of relatively small occupational cohorts. The Sarasua et al. (2000) study included a cross-sectional analysis of abundance of total lymphocytes, B-cells, NK cells, and $CD4^+$ and $CD8^+$ T-cell phenotypes in individuals (n = 433), age 16 to 75 years. Associations were not

found between blood Pb concentration and either B-cell or T-cell abundance, after adjusting for age, sex, and study site (of four mining/smelting sites). The study did detect significant associations among these variables in infants and children (see above discussion of cellular immunity outcomes in children). However, all three occupational studies found significant associations between increasing blood Pb concentrations and changes in abundance of circulating T-cells with either no effect or an increasing B-cell abundance (Fischbein et al., 1993; Pinkerton et al., 1998; Sata et al., 1997). The strengths of the Pinkerton et al. (1998) study have been described previously with respect to outcome measures for humoral immunity. The study included male smelter workers (n = 145, mean blood Pb 39 μg/dL; range 15–55) and hardware workers (n = 84, mean <2 μg/dL, range <2–12). Covariate-adjusted significant outcomes were an increase in B-cell ($CD19^+$) abundance (% and number) and increases in $CD4^+CD45RA^+$ cell abundance (%, number) in association with increasing blood Pb concentration. Covariate-adjusted mean levels of monocytes (%), and T-cells (% $D4^+CD8^+$, $CD8^+CD56^+$) were lower in Pb workers compared to the reference group.

The Fischbein et al. (1993) study examined a small group of firearms instructors (n = 51) and age-matched reference subjects (n = 36). Fifteen of the instructors had blood Pb levels ≥25 μg/dL (mean 31.4, SD 4.3), the mean of the remaining 21 subjects was 4.6 μg/dL (SD 4.6). Mean blood Pb concentration of the reference group was reported as <10 μg/dL. Increasing blood Pb concentration was significantly associated with decreasing covariate-adjusted T-cell ($CD4^+$) abundance (Figure 6-19). Covariate-adjusted T-cell ($CD3^+$% and number, $CD4^+$% and number, $CD4^+CD8^+$ number) abundance was significantly lower and B-cell ($CD20^+$ cells % and number) abundance was higher in the instructors than in the reference group.

The Sata et al. (1998) study included male Pb stearate manufacture workers (n = 71) and a nonexposed reference group (n = 28). Mean blood Pb concentration was 19 μg/dL (range 7-50) in the Pb workers (reference group blood Pb concentration not reported). Categorical covariate-adjusted Pb exposure classification (exposed, not exposed) was significantly associated with lower T-cell ($CD3^+CD45RO^+$) number. Lead workers, relative to the reference group, had significantly lower covariate-adjusted mean $CD3^+CD45RO^+$ number and higher $CD8^+$ cells (%).

The above observations of decreasing T-cell abundance in association with Pb exposure, as assessed from blood Pb concentrations, is supported by results of several smaller cross-

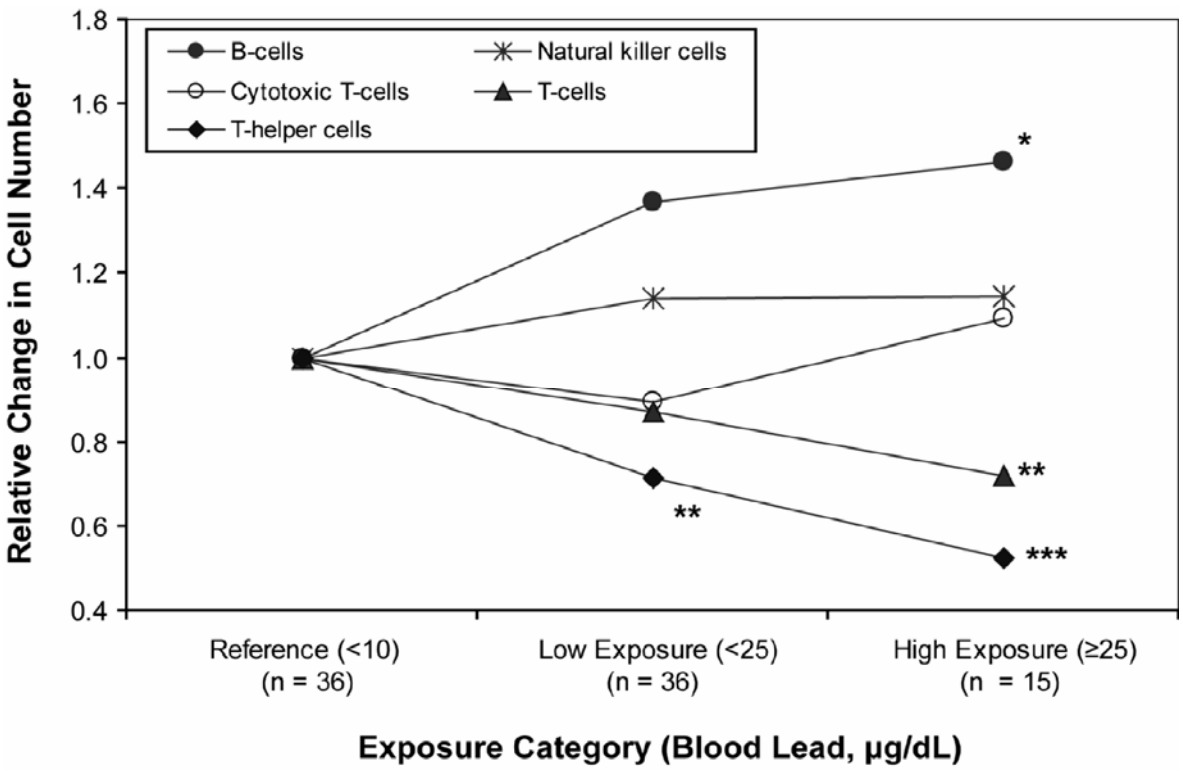

Figure 6-19. Relationship between lead exposure and T- and B-cell abundances in firearms instructors. Shown are relative changes in absolute cell numbers compared to the reference group. Comparisons of exposed relative to the reference group are shown as: * for $p < 0.05$; ** for $p < 0.01$; and * for $p < 0.002$. Receptor phenotypes assayed were: T-cells, CD3+; T-helper cells, CD4+; cytotoxic T-cells, CD8+; natural killer cells, CD16+; B-cells, CD20+. The CD4+/CD8+ ratio (not shown) was significantly lower in both the low exposure (1.38 [SD 0.5], $p < 0.002$) and higher exposure group (0.95 [SD 0.5], $p < 0.002$), compared to the reference group (1.95 [SD 0.66]).**

Source: Fischbein et al. (1993).

sectional studies, including Basaran and Ündeğer (2000), Coscia et al. (1987), and Ündeğer et al. (1996), as well as a clinical study in which T-cell and NK cell abundance was found to increase after CaEDTA chelation therapy of a Pb smelter worker (Sata et al., 1997). Lower serum levels of the cytokines that function in the regulation of cellular immune responses, including IL-1β and IFN-γ, in Pb workers compared to nonexposed subjects have also been observed (Yücesoy et al., 1997a).

6.8.5 Lymphocyte Function

Studies conducted in animal models have found mixed effects of Pb on mitogen-induced lymphocyte activation, promoting expansion of some types of lymphoid populations, while suppressing others (see Section 5.9.5). Lead promotes the activation of Th2-type lymphocytes and suppresses Th1 type lymphocytes; it also shifts the balance in the production of cytokines, decreasing Th1 cytokines (e.g., IFN, IL-12) and increasing production of Th2 cytokines (e.g., IL-4, IL-6, IL-10). The above findings are somewhat echoed in the overall findings from epidemiologic studies, with mixed outcomes when proliferation of peripheral lymphocytes was the outcome measured, whereas, Pb preferentially stimulated Th2 cytokine production and suppressed Th1 cytokine production when human peripheral lymphocytes were exposed to Pb in vitro.

Several studies (all of adults) have examined associations between Pb exposure in adults and lymphocyte activation, assessed as a proliferative response to mitogens and/or antigens (Alomran and Shleamoon, 1988; Cohen et al., 1989; Fischbein et al., 1993; Kimber et al., 1986; Mishra et al., 2003; Pinkerton et al., 1998; Queiroz et al., 1994b). Results of these have been mixed. Three studies found no significant associations between blood Pb concentrations in Pb workers and lymphocyte proliferative response to activating agents (Kimber et al., 1986; Pinkerton et al., 1998; Queiroz et al., 1994b). Four studies found decreasing proliferative response with increasing blood Pb concentration (Alomran and Shleamoon, 1988; Cohen et al., 1989; Fischbein et al., 1993; Mishra et al., 2003). The Alomran and Shleamoon (1988), Cohen et al. (1989), Mishra et al. (2003), and Queiroz et al. (1994b) studies, which found significant Pb associations, included subjects who had relatively high blood Pb levels (>60 μg/dL) compared to the Kimber et al. (1986) and Pinkerton et al. (1998) studies. The inclusion of subjects with higher Pb concentrations may have contributed to the differences in outcomes.

As noted in the previous section, the Fischbein et al. (1993) and Pinkerton et al. (1998) studies are particularly noteworthy because of the strengths of the cohort selection and the data analyses which attempted to account for potential confounders. Also, these are the only reported studies that examined antigen-specific lymphocyte activation in humans. Mean blood Pb levels in the two studies were similar: 31 μg/dL (SD 4) in the Fischbein et al. (1993) study and 39 μg/dL (range 15–55) in the Pinkerton et al. (1998) study. Both studies found no significant associations between blood Pb concentration and antigen-specific lymphocyte proliferation,

assessed in the Pinkerton et al. (1998) study with tetanus toxoid as the antigen and in the Fischbein et al. (1993) study with staphylococcus aureus as the antigen. However, the Fischbein et al. (1993) study also measured mitogen-induced lymphocyte proliferation (induced with PHA or PWM) and found a significantly lower proliferative response to the mitogens in association with Pb exposure. This study also found a significant association between increasing blood Pb concentration and decreasing proliferative response in mixed lymphocyte cultures (i.e., proliferative response of lymphocytes from exposed subjects when incubated with inactivated lymphocytes from a reference subject).

Inorganic Pb has been shown by in vitro studies to perturb several aspects of lymphocyte function when introduced into primary isolates of human blood monocytes. Activated lymphocytes show altered lysosomal enzyme secretion and altered expression and secretion of cytokines (Bairati et al., 1997; Guo et al., 1996a; Hemdan et al., 2005). Lymphocytes activated with *Salmonella enteritidis* or with monoclonal antibodies of CD3, CD28 and CD40, and exposed to inorganic Pb had suppressed expression of T-helper cell type T_H-1 cytokines, interferon (IFN-γ), interleukin (IL-1β), and tumor necrosis factor (TNF-α), whereas activation by CD antibodies increased secretion of T_H-2 cytokines, IL-5, IL-6, and IL-10 (Hemdan et al. 2005). Inorganic Pb also activates transcription factor NK-κβ in $CD4^+$ cells (Pyatt et al., 1996), an important regulator of T-cell activation, and increases expression of MHC class II surface antigens (HLA-DR), an important surface antigen in the $CD4^+$ response to exogenous antigens (Guo et al., 1996b). Lead increases antibody production in cultured human B-cells (McCabe and Lawrence, 1991). These observations suggest that Pb may perturb cellular immune function through a variety of mechanisms.

6.8.6 Phagocyte (Macrophage and Neutrophil) Function

Animal studies and in vitro models have shown that Pb can modulate macrophages into a hyperinflammatory phenotype, with increased production of proinflammatory cytokines TNF-α and IL-6, increased release of reactive oxygen intermediates and prostaglandins, and, conversely, depressed production of nitric oxide (see Section 5.9.6). Epidemiologic studies have found associations between blood Pb concentrations and modified activation of macrophages in children whose blood Pb levels ranged from 4 to 50 μg/dL (Pineda-Zavaleta et al., 2004). Consistent with the above experimental observations, outcomes have included decreased

stimulated nitric oxide release and increased superoxide anion production. In addition, studies have observed suppressed PMNL chemotaxis in association with occupational exposures that resulted in blood Pb concentrations of 12 to 90 μg/dL (Bergeret et al., 1990; Queiroz et al., 1994a, 1993).

Pineda-Zavaleta et al. (2004) examined mitogen (PHA)- and cytokine (INFγ)-induced activation of blood monocytes collected from 65 children (age range 6 to 11 years) who resided near an active Pb smelter. Mean blood Pb concentrations of subjects at three schools located 8,100 meters, 1,750 meters, and 650 meters from the smelter were: 7.0 μg/dL (range 3–25), 21 μg/dL (range 11–49), and 30 μg/dL (range 10–48), respectively. Endpoints measured included nitric oxide and superoxide anion production, a response generally attributed to activated macrophages. Increasing blood Pb concentration was significantly associated with decreasing PHA-induced nitric oxide production and increasing INFγ-induced superoxide anion production. The mitogen, PHA, activates macrophages indirectly through activation of lymphocytes, whereas INFγ, a cytokine released from CD44 (T_H1) cells, directly activates macrophages. Thus, one interpretation of this outcome is that Pb suppressed T-cell mediated macrophage activation and stimulated cytokine-induced macrophage activation.

Possible associations between occupational Pb exposure and PMNL chemotaxis and phagocytic activity have been explored in several small cross-sectional studies. Consistent findings are significantly reduced chemotactic response and phagocytic activity (i.e., respiratory burst, luminal uptake) in Pb workers compared to reference groups. The largest study is that of Queiroz et al. (1993, 1994a) which evaluated PMNL function in several (possibly overlapping) cohorts of Pb battery manufacture workers (n = 60). Blood Pb concentrations in the study groups ranged from 12 to 90 μg/dL. PMNL chemotaxis and lytic activity were significantly lower in the Pb workers compared to the reference group. Bergeret et al. (1990) assessed PMNL chemotaxis and phagocytosis in a group of battery smelting workers (n = 34) and in a group of reference subjects (n = 34) matched to the Pb worker group by age, sex, ethnic origin, smoking and alcohol consumption habits, and intake of antibiotics and NSAIDs. Mean blood Pb levels were 71 μg/dL (SD 18) in the Pb workers and 9 μg/dL (SD 4) in the reference group. Significantly lower PMNL chemotactic response to FMLP and phagocytic response in opsonized zymosan were significantly lower in the Pb workers than in the reference group. Lead

introduced into primary cultures of human PMNLs suppressed chemotaxis and phagocytosis (Governa et al., 1987).

6.8.7 Summary of the Epidemiologic Evidence for the Effects of Lead on the Immune System

Studies conducted in animals and in vitro experimental models have shown that Pb can alter immune system function (see Section 5.9). Lead appears to target, preferentially, macrophages and T lymphocytes; although, effects on B cells and neutrophils have also been reported. The prominent effects are largely on immune system function, rather than overt cytotoxicity to lymphoid tissues. Lead suppresses Th1-dependent responses (e.g., delayed type hypersensitivity) and production of Th1 cytokines and shifts the Th1/Th2 balance towards Th2 responses; increases the production of IgE and Th2 cytokines (e.g., Il-4); and stimulates macrophages into a hyperinflammatory state. These types of functional changes have not been rigorously evaluated in human epidemiologic studies, which have relied, for the most part, on changes in lymphocyte abundance or circulating immunoglobulin levels, as the main outcomes for assessing status of cellular immune systems. The above outcomes may be relatively insensitive to for detecting disturbances in humoral or cellular immune function. Few studies have attempted to examine associations between Pb exposure and integrated immune function (e.g., host resistance, hypersensitivity, autoimmunity) and current epidemiologic evidence for associations between Pb exposure and compromised immune function in humans, reflected in risk of asthma or infections, is not compelling, but these studies may not have been adequate to address this.

- Several epidemiological studies have examined possible associations between Pb exposures and various indices of humoral and cellular immune status. Findings from these studies suggest that Pb exposure (as reflected in blood Pb concentration) may be associated with changes in serum immunoglobulin levels; perturbation of peripheral lymphocyte phenotype profiles, including decreases in peripheral blood T-cell abundance and changes in T-cell:B-cell abundance ratios; modulation of lymphocyte activation (increased stimulated lymphocyte release of reactive oxygen intermediates and suppressed production of nitric oxide; increased production of Th2 cytokines and suppression of Th1 cytokines); and suppression of neutrophil chemotaxis and phagocytosis. Observations of increased circulating levels of IgE, increased release of reactive oxygen intermediates and suppressed production of nitric oxide in peripheral lymphocytes are of particular interest in that such effects have been consistently observed in studies conducted in animals and in vitro model.

- Studies in children have consistently found significant associations between increasing blood Pb concentration and increasing serum IgE. These effects have been observed at blood Pb concentrations <10 µg/dL. Findings of studies of adults have been mixed with significant associations between blood Pb (>30 µg/dL) and serum immunoglobulin levels and no association in a study group in which blood Pb concentrations were <10 µg/dL.

- Studies in children have also found significant associations between increasing blood Pb concentration and decreases in T-cell abundance, with corresponding increases in B-cell abundance. These effects have been observed in children whose blood Pb concentrations were <10 µg/dL, although not all studies have found such associations at higher blood Pb concentrations (e.g., in the 10 to 45 µg/dL range).

- Studies of occupational Pb exposures have also found associations between increasing blood Pb concentration and decreasing T-cell abundance. Effects were observed in association with blood Pb concentrations <25 µg/dL and in populations whose blood Pb concentrations ranged from ~7 to 55 µg/dL.

- Studies of lymphocyte and phagocyte (i.e., macrophage, neutrophil) function have found associations between blood Pb concentrations and modulation of the activation of lymphocytes and macrophages in children whose blood Pb concentrations ranged from 4 to 50 µg/dL, suppressed PMNL chemotaxis in association with occupational exposures that resulted in blood Pb concentrations of 12 to 90 µg/dL, and suppressed mitogen-induced activation of peripheral lymphocytes in adults in association with occupational exposures that resulted in blood Pb levels that ranged from 15 to 55 µg/dL. Consistent with observations made in animal models, Pb exposures in vitro suppressed production of Th1 cytokines and stimulated production of Th2 cytokines in isolates of peripheral lymphocytes.

6.9 EFFECTS OF LEAD ON OTHER ORGAN SYSTEMS

6.9.1 Biochemical Effects of Lead

6.9.1.1 Summary of Key Findings of the Biochemical Effects of Lead from the 1986 Lead AQCD

The 1986 Lead AQCD provided an extensive discussion of the effects of Pb on heme biosynthesis and on quantitative relationships between exposure and effects in humans. Lead interferes with heme synthesis by inhibiting the enzymes ALAD and ferrochelatase. As a consequence, heme biosynthesis decreases, relieving the rate-limiting enzyme of the heme synthesis pathway, δ-aminolevulinic synthetase (ALAS), from negative feedback inhibition by heme (Figure 6-20). The outcomes of decreased activity of ALAD and ferrochelatase, and increased activity of ALAS are increased urinary excretion of coproporphyrin (CP) and

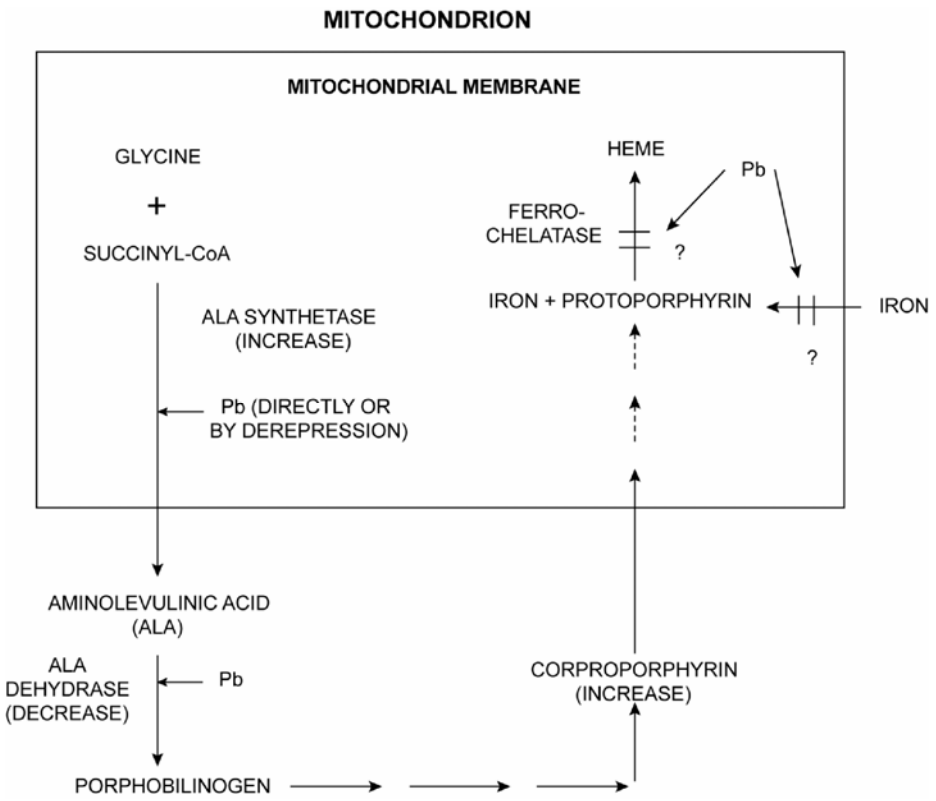

Figure 6-20. Effects of lead on heme biosynthesis.

Source: Derived from EPA (1986).

δ-aminolevulinic acid (ALA), increased level of ALA in blood plasma, and increased erythrocyte protoporphyrin (EP) levels.

Associations between Pb exposure and blood ALAD activity and EP levels, and urinary ALA and CP excretion have been studied extensively in adults and children, and quantitative relationships between exposure and effect are well understood. Much of this information was available prior to completion of the 1986 Lead AQCD and is discussed in detail in that criteria document (e.g., Alessio et al., 1976; Hernberg et al., 1970; Lilis et al., 1978; Piomelli et al., 1982; Roels et al., 1979; Selander and Cramér, 1970; Valentine et al., 1982). Numerous studies published since the 1986 AQCD provide additional support for the Pb concentration-response relationships in humans described in the 1986 AQCD. The most pertinent new studies are summarized in Annex Tables AX6-9.1 and AX6-9.2. The studies that provide the strongest basis

for empirically-derived expressions relating blood Pb concentration, blood ALAD activity, urinary ALA, and EP are listed in Table 6-7 and are discussed below.

Since completion of the 1986 Lead AQCD, a literature has developed on the effects of Pb on serum and blood lipids, including cholesterol levels and indications of oxidative stress, in the form of lipid peroxides, depletion of erythrocyte reduced glutathione (GSH), and production of reactive oxygen species (ROS). These studies also are summarized in Annex Tables AX6-9.1 and AX6-9.2, and key findings are discussed below.

6.9.1.2 Heme Biosynthesis

6.9.1.2.1 ALAD Inhibition

Numerous studies published since the 1986 AQCD have explored associations between Pb exposure and inhibition of ALAD activity, as assessed from measurements of blood ALAD activity (Gurer-Orhan et al., 2004; Kim et al., 2002; Lee et al., 2000; Makino et al., 1997; Roels and Lauwerys, 1987; Schuhmacher et al., 1997), or urinary ALA excretion (Gennart et al., 1992; Oishi et al., 1996; Schuhmacher et al., 1997; Wildt et al., 1987; Soldin et al., 2003). Quantitative estimates derived from the larger, more recent studies are presented in Table 6-7. Blood Pb concentration is inversely correlated with the log of blood ALAD activity and log of urinary ALA and quantitative estimates of the change in blood. ALAD activity per unit change in blood Pb concentration are consistent across studies (observed blood Pb range 5 to 150 µg/dL). Halving of blood ALAD activity occurs with an increase in blood Pb concentration of ~20 µg/dL in both children (Roels and Lauwerys, 1987) and adults (Morita et al., 1997). These estimates are consistent with earlier studies of adults (e.g., Hernberg et al., 1970) and children (e.g., Alessio et al., 1976, 1977), discussed in the 1986 AQCD. Greater variability is apparent in estimates of the change in urinary ALA per unit change in blood Pb concentration (Table 6-7). This may be related, in part, to gender-heterogeneity in the relationship. Roels and Lauwerys (1987) estimated that urinary ALA doubles in association with a 20 µg/dL increase in blood Pb concentration in females and 50 µg/dL in males. In a much larger study (Oishi et al., 1996), an analysis that combined data from males (n = 253) and females (n = 165) found that a doubling of urinary ALA occurred in association with a 13.7 µg/dL increase in blood Pb concentration. Urinary ALA excretion increases as a linear function of plasma ALA concentration (Oishi et al., 1996); thus, the gender heterogeneity for the blood Pb-urinary ALA relationship may derive

Table 6-7. Blood Lead–Response Relationships for Heme Synthesis Biomarkers in Adults and Children

Study	n	Age	Blood Lead (µg/dL) Range	Regression Equation (r)	Blood Lead Change (µg/dL) Predicted to Halve or Double Effect Biomarker
ALAD Activity Decrease					
Roels and Lauwerys (1987)	143	10–13 yr	5–41	log[ALAD] = 1.864–0.015[blood Pb] (r = 0.87)	20.1
Alessio et al. (1976, 1977)	169	Adult (M)	15–150	log[ALAD] = 3.73–0.031[blood Pb] (r = 0.87)	22.4
Hernberg et al. (1970)	158	Adult (M, F)	5–95	log[ALAD] = 2.274–0.018[blood Pb] (r = 0.90)	16.1
Morita et al. (1997)	58	Adult (M)	2–82	log[ALAD] = 1.8535–0.00971[blood Pb] (r = 0.76)	20.1
Urinary ALA Increase					
Roels and Lauwerys (1987)	37	10–13 yr	20–41	log[ALAU] = 0.94+0.11[blood Pb] (r = 0.54)	20.9
Alessio et al. (1976, 1977)	316	Adult (M)	10–150	log[ALAU] = 1.25+0.014[blood Pb] (r = 0.62)	49.5
Gennart et al. (1992)	183	Adult (M, F)	4–75	log[ALAU] = 0.37+0.008[blood Pb] (r = 0.64)	37.6
Oishi et al. (1996)	418	Adult (M, F)	10–99	log[ALAU] = −0.387+0.022[blood Pb] (r = 0.71)	13.7
Selander and Cramér (1970)	150	Adult (M, F)	6–90	log[ALAU] = −1.0985+0.157[blood Pb] (r = 0.74)	19.2
Roels and Lauwerys (1987)	39	Adult (M)	10–60	log[ALAU] = 0.37+0.006[blood Pb] (r = 0.41)	50.2
Roels and Lauwerys (1987)	36	Adult (F)	7–53	log[ALAU] = 0.15+0.015[blood Pb] (r = 0.72)	20.1

Table 6-7. (cont'd). Blood Lead–Response Relationships for Heme Synthesis Biomarkers in Adults and Children

Study	n	Age	Blood Lead (µg/dL) Range	Regression Equation (r)	Blood Lead Change (µg/dL) Predicted to Halve or Double Effect Biomarker
EP Increase					
Marcus and Schwartz (1987)	1,677	2-6	6-65	Nonlinear kinetic model	20 –40[a]
Piomelli et al. (1982)	2,002	2–12	2–98	log[EP] = 1.099+0.016[blood Pb] (r = 0.509)	18.8
Roels and Lauwerys (1987)	51	10–13	15–41	log[EP] = 1.321+0.025[blood Pb] (r = 0.73)	12.0
Soldin et al. (2003)	4,908	0–17	<1–103	EP = –0.0015[blood Pb]^3+0.1854[blood Pb]^2–2.7554[blood Pb]+30.911 (r = 0.999)	20.6
Alessio et al. (1976, 1977)	95	Adult (M)	10–90	log[EP] = 0.94+0.0117[blood Pb]	25.7
Alessio et al. (1976, 1977)	93	Adult (F)	10–70	log[EP] = 1.60+0.0143[blood Pb]	21.1
Gennart et al. (1992)	183	Adult (M)	4–75	log[EP] = 0.06+0.019[blood Pb] (r = 0.87)	15.8
Roels and Lauwerys (1987)	39	Adult (M)	10–60	log[EP] = 1.41+0.014[blood Pb] (r = 0.74)	21.1
Roels and Lauwerys (1987)	36	Adult (F)	7–53	log[EP] = 1.23+0.027[blood Pb] (r = 0.81)	11.1
Wildt et al. (1987)	851	Adult (M)	10–80	log[EP] = 1.21+0.0148[blood Pb] (r = 0.72)	20.3
Wildt et al. (1987)	139	Adult (F)	10–80	log[EP] = 1.48+0.0113[blood Pb] (r = 0.56)	20.6

ALA, δ-aminolevulinic acid; ALAD, δ-aminolevulinic acid dehydratase; ALAU, urinary δ-aminolevulinic acid; EP, erythrocyte protoporphyrin; F, female; M, male.

[a]Approximately 20 µg/dL at low transferrin saturation (<31%), approximately 40 µg/dL at higher transferrin saturation (>31%).

from a gender difference in the effect of Pb on plasma ALA concentration or from differences in renal plasma clearance of ALA.

6.9.1.2.2 ALAD Polymorphism

ALAD is a polymorphic enzyme with two alleles (ALAD1 and ALAD2) and three genotypes: ALAD 1-1, ALAD 1-2, and ALAD 2-2 (Battistuzzi et al., 1981). The corresponding phenotypes appear to have nearly identical catalytic properties (Battistuzzi et al., 1981). The predominant genotype is ALAD 1-1 which has a prevalence of ~90% (Astrin et al., 1987; Battistuzzi et al., 1981; Hsieh et al., 2000; Shen et al., 2001). A significantly higher percentage ($p = 0.03$) of erythrocyte Pb was bound to ALAD in carriers of the ALAD2 allele (84%) compared to carriers of the ALAD1 allele (81%); however, no differences were evident in the distribution of Pb between erythrocytes and plasma (Bergdahl et al., 1997), and there is no evidence that the ALAD genotype confers different sensitivity to inhibition of heme biosynthesis (Hsieh et al., 2000; Perez-Bravo et al., 2004; Schwartz et al., 1997; Süzen et al., 2003).

6.9.1.2.3 Ferrochelatase Inhibition

Lead inhibition of ferrochelatase results in an accumulation of protoporphyrin IX in erythrocytes (EP, also referred to as zinc protoporphyrin, or ZPP). Numerous studies have examined relationships between blood Pb concentration and EP levels in adults and children. Quantitative estimates based on the most pertinent studies are presented in Table 6-7. Results across these studies are similar (observed blood Pb range: <1 to 103 μg/dL). In both children and adults (males and females), a doubling of EP levels occurs in association with an increase in blood Pb concentration of ~20 μg/dL (Marcus and Schwartz 1987; Piomelli et al., 1982; Soldin et al., 2003; Wildt et al., 1987). However, the relationship between blood Pb concentration and EP level is not linear (Marcus and Schwartz, 1987; Soldin et al., 2003). The slope of the blood Pb concentration range over which a change (threshold) in EP occurs is relatively small and appears to extend to ~20 μg/dL in iron replete children but decreases with increasing iron deficiency (Marcus and Schwartz, 1987). A pronounced gender difference in the relationship between EP and blood Pb concentration was observed by Roels and Lauwerys (1987) which was not observed in the much larger study of Wildt et al. (1987).

Sakai et al. (2000) examined the relationship between ALAD genotypes and disturbances in heme biosynthetic pathway upon Pb exposure in 192 males occupationally exposed to Pb and 125 controls. ALAD1 homozygotes had significantly higher EP levels compared to ALAD2 carriers at blood Pb values >20 μg/dL, suggesting that they might be more susceptible to disturbances in heme metabolism caused by Pb exposure.

Inhibition of ferrochelatase also gives rise to an increase in urinary coproporphyrin, with a similar relationship to blood Pb concentration; a doubling of urinary EP occurs in association with an increase in urinary coproporphyrin of ~20 μg/dL (Alessio et al., 1976).

6.9.1.3 Effects on Blood Lipids: Cholesterol

Associations between occupational exposure to Pb and changes in blood lipid composition have been observed. These include increased levels of lipid peroxides in blood and/or serum (Ito et al., 1985; Jiun and Hsien, 1994; Sugawara et al., 1991) and increased serum levels of total and HDL cholesterol (Kristal-Boneh et al., 1999). Increased levels of glucose-6-phosphate dehydrogenase (G6PD) in erythrocytes have also been observed in Pb workers (Cocco et al., 1995; Gurer-Orhan et al., 2004).

Kristal-Boneh et al. (1999) measured serum total, HDL, and LDL cholesterol, and triglycerides in a group of male battery manufacture workers. Covariate-adjusted serum total-cholesterol and HDL cholesterol levels were 6% and 12% higher, respectively, in Pb workers (n = 56, mean blood Pb 42 μg/dL, SD 15) compared to reference group (mean blood Pb: 2.7 μg/dL). Increasing blood Pb concentration was significantly associated with increasing covariate-adjusted total cholesterol and HDL cholesterol. A similar outcome was found in a larger study (Ito et al., 1985) of male steel workers (n = 712, blood Pb range 5–62 μg/dL). When stratified by age, total and HDL cholesterol levels in serum were 3.6% and 7.5% higher, respectively, in Pb workers in the age range 40 to 49 years, compared to corresponding strata of the office workers (n = 155). Although a smaller study, the Kristal-Boneh et al. (1999) study considered a larger set of potential covariables (e.g., dietary fat, cholesterol, and calcium intakes, sport activities, alcohol consumption, cigarette smoking).

Oxidative changes in blood lipids (e.g., increased levels of lipid peroxides and malondialdehyde levels) as well as decreased levels of erythrocyte superoxide dismutase (SOD), catalase, G6PD, and GSH peroxidase, indicative of increased oxidative stress, have been

observed in Pb workers, in comparison to reference groups (Ito et al., 1985; Jiun and Hsien, 1994; Solliway et al., 1996; Sugawara et al., 1991). However, none of these studies have developed concentration-response relationships that take into account potential confounders. The largest study is that of (Ito et al., 1985), described above. When stratified by age, serum lipoperoxide levels were 16% higher in the Pb workers in the age range 40 to 49 years, compared to corresponding strata of the reference group. Serum lipoperoxide levels also appeared to increase as blood Pb increased above 30 μg/dL, while erythrocyte SOD appeared to decrease with increasing blood Pb concentration (a statistical evaluation was not reported).

Evidence for increased oxidative stress (increased reactive oxygen species) in lymphocytes of Pb workers has also been reported (Fracasso et al., 2002). Peripheral lymphocytes collected from battery manufacture workers (n = 37, mean blood Pb: 40 μg/dL) exhibited increased DNA strand breaks, higher production of ROS and lower GSH levels compared to a reference group of office workers (n = 29, mean blood Pb 4 μg/dL). The covariate-adjusted odds ratios (exposed versus not exposed) were 1.069 (95% CI: 1.020, 1.120) for increased DNA strand breaks and 0.634 (95% CI: 0.488, 0.824) for lower GSH levels.

6.9.2 Effects of Lead on the Hematopoietic System

6.9.2.1 Summary of Key Findings of the Effects of Lead on the Hematopoietic System from the 1986 Lead AQCD

The 1986 Lead AQCD concluded that Pb decreases heme production and shortens erythrocyte survival; both effects contributing to Pb-induced anemia in children and adults, which becomes evident in children at blood Pb concentrations ≥40 μg/dL and, in adults, ≥50 μg/dL. The 1986 Lead AQCD also concluded that effects of Pb on blood hemoglobin level extend below 50 μg/dL, with effects detected in Pb workers at blood Pb concentrations <25 μg/dL (Baker et al., 1979; Grandjean, 1979). More recent epidemiologic studies, summarized below, provide additional information on concentration-response relationships for hematopoietic effects of Pb. The studies support the conclusion that clinical anemia can occur in children in association with blood Pb levels >40 μg/dL (Schwartz et al., 1990). The newer studies suggest that perturbation of erythropoiesis, indicated by changes in serum erythropoietin, occurs in association with blood Pb concentrations <40 μg/dL and in the absence of detectable changes in blood hemoglobin levels or hematocrit. Details regarding the design of these studies

and outcomes are presented in Annex Tables AX6-9.3 and AX6-9.4. Outcomes of the most pertinent studies are discussed below.

6.9.2.2 Blood Hemoglobin Levels

Several studies reported since the completion of the 1986 Lead AQCD have explored associations between Pb exposure and blood hemoglobin levels in children and adults. Consistent findings have been a lack of discernable depression of blood hemoglobin levels in study populations whose mean blood Pb concentrations were ≤40 µg/dL (Table 6-8). Of note is the findings relating patella bone Pb to both blood hemoglobin levels and hematocrit.

The Kosovo prospective study of pregnancy outcomes is one of the largest epidemiologic evaluations of associations between Pb exposure and blood hemoglobin levels in infants and children (Graziano et al., 2004; Factor-Litvak et al., 1998, 1999). The study included pregnant women (n = 1502) and their children (n = 311) who resided in one of two regions of Kosovo, Yugoslavia; one was heavily impacted by Pb industries (high-Pb area), the other had relatively little Pb contamination (low-Pb area). Mean blood Pb concentrations of children (measured at birth and at intervals to 12 years of age) ranged from 30 to 40 µg/dL in the high-Pb area and 6 to 9 µg/dL in the low-Pb area. Mean blood hemoglobin levels in the low-Pb and high-Pb children, measured at 4.5, 6.5, 9.5, and 12 years of age, were not significantly different. These findings are consistent with those from a smaller cross-sectional study (n = 89; blood Pb range 2 to 84 µg/dL, 84% <35 µg/dL) that also found no association between blood Pb concentration and blood hemoglobin levels (Liebelt et al., 1999). Results from these two studies suggest that, in the absence of iron deficiency, Pb exposures that result in blood Pb levels <40 µg/dL do not produce detectable changes in blood hemoglobin levels in children.

Associations between Pb exposure and blood hemoglobin levels in adults have been examined in numerous epidemiological studies (Froom et al., 1999; Gennart et al., 1992; Horiguchi et al., 1991; Hu et al., 1994; Makino et al., 1997; Poulos et al., 1986; Romeo et al., 1996; Solliway et al., 1996). The Graziano et al. (1990) and Makino et al. (1997) studies warrant particular attention because of the design (longitudinal), relatively large size (>1000 subjects), and relatively low blood Pb levels of the subjects (<40 µg/dL). Both studies support the general conclusion that blood hemoglobin levels are not depressed in association with blood Pb concentrations <40 µg/dL. In the Kosovo prospective study, no discernable effect of Pb on

Table 6-8. Summary of Results of Selected Studies of Associations Between Lead Exposure and Blood Hemoglobin Levels

Study	Subjects	n[a]	Blood Lead (µg/dL)		Blood Hemoglobin	Comment
			Mean (SD)	Range		
Children						
Graziano et al. (2004)	Ages: 4.5–12 yr	311	6–9, 31–39[b]	3–70	o	+ erythropoietin
Liebelt et al. (1999)	Ages: 1–6 yr	86	18[c]	2–84	o	− erythropoietin
Adults						
Graziano et al. (1990)	Pregnant women	1,502	5, 17[d]	2–43	o	− erythropoietin
Hu et al. (1994)	Male carpenters	119	8	2–25	o	− in association with patella bone lead
Makino et al. (1997)	Male vinyl chloride stabilizer workers	1,573	13	1–39	+	(+) 1 g/dL per 10 µg/dL blood lead
Solliway et al. (1996)	Male battery workers	100	10	23–63	o	− red blood cell count
Gennart et al. (1992)	Battery workers	183	51 (8)	40–70	−	− hematocrit
Horiguchi et al. (1991)	Male lead refinery workers	40	54 (16)	NR	−	− hematocrit
Poulos et al. (1986)	Male lead workers	160	18–27 (5)[e]	NR	−	− hematocrit

−, decrease; +, increase; o, no effect; NR, not reported.

[a] Total number of subjects (including reference group)
[b] Range of means of low and higher exposure groups
[c] Median
[d] Mean of low- and high-exposure groups
[e] Range of group means (standard deviation estimated for up range based on reported standard error)

maternal blood hemoglobin levels was evident from a comparison of the high-Pb exposure group (mean blood Pb 17 μg/dL, range 7–43) with the low-Pb exposure group (mean blood Pb 5.1 μg/dL, range 2–11). Makino et al. (1997) found a positive association between increasing blood Pb concentration and increasing blood hemoglobin levels in a longitudinal survey of adult males (n = 1,573) who worked in pigment or vinyl chloride stabilizer manufacture (mean blood Pb 13 μg/dL, range 1–39). A simple linear regression model predicted a 10 μg/dL increase in blood hemoglobin per 10 μg/dL increase in blood Pb concentration (typical level 10–20 μg/dL).

Two other cross-sectional studies are also notable, because of design considerations and/or blood Pb concentration ranges of the subjects. Solliway et al. (1996) observed no differences in mean blood hemoglobin levels in a comparison of adult male battery manufacture workers (n = 34; mean blood Pb 41 μg/dL, range 23–63) and a matched reference group (n = 56; mean blood Pb 7 μg/dL, range 1–13). Hu et al. (1994) conducted a cross-sectional assessment of adult male carpentry workers (n = 119) whose blood Pb levels were ≤25 μg/dL. Blood hemoglobin was not significantly associated with blood Pb concentration. Of note, however, was the finding that increasing patella bone Pb was significantly associated with decreasing blood hemoglobin levels. Covariate-adjusted blood hemoglobin levels were predicted to decrease by 1.1 g/dL per 37 μg/g increase (mean of first and fourth quartiles) in patella bone Pb.

Studies of Pb workers whose blood Pb levels were higher than in the studies noted above have, in general, found lower blood hemoglobin levels in association with increasing blood Pb concentrations; these include Gennart et al. (1992) with a blood Pb range of 40 to 70 μg/dL, Horiguchi et al. (1991) with a mean blood Pb level of 54 μg/dL (SD 16), and Poulos et al. (1986) with mean blood Pb range of 21 to 27 μg/dL. In the latter study (Poulos et al., 1986), blood hemoglobin levels decreased by 0.6 to 0.9 g/dL per 10 μg/dL increase in blood Pb (simple linear regression) in adult males. Analyses or adjustments for potential covariables were not reported for these studies.

6.9.2.3 Erythrocyte Volume and Number

Schwartz et al. (1990) conducted a concentration-response analysis of data collected at the Bunker Hill smelter site in Idaho in 1974, shortly after the failure of the smelter bag house resulted in extensive contamination of the surrounding area with uncontrolled smelter emissions. This analysis is unique in that it collected hematocrit measurements in children (n = 579, age

range 1 to 5 years) who had relatively high blood Pb levels (range 11–164 μg/dL, ~40% exceeded 40 μg/dL). A logistic model relating blood Pb concentration and age to hematocrit predicted a 10% decrease in hematocrit (from 39.5 to 35.5%) in association with blood Pb concentrations of 85, 115, and 145 μg/dL at ages 1, 3, and 5 years, respectively (Figure 6-21). A 10% probability of anemia (hematocrit <35%) was predicted in association with a blood Pb concentration of ~20 μg/dL at age 1 year, 50 μg/dL at age 3 years, and 75 μg/dL at age 5 years (Figure 6-21).

Numerous studies of associations between Pb exposure and erythrocyte volume (e.g., hematocrit) or number have been reported in adults (Gennart et al., 1992; Horiguchi et al., 1991; Hsiao et al., 2001; Hu et al., 1994; Makino et al., 1997; Osterode et al., 1999; Poulos et al., 1986; Solliway et al., 1996). The Hu et al. (1994) and Makino et al. (1997) studies examined groups of workers that had blood Pb concentrations that were relatively low, compared to other studies, and found either no association or weak association between blood Pb concentration and hematocrit and/or erythrocyte number. The Hu et al. (1994) cross-sectional study of carpentry workers (n = 119, blood Pb concentration range 2–25 μg/dL) found no association between blood Pb concentration and hematocrit; however, increasing patella bone Pb was associated with a significant decrease in hematocrit. Covariate-adjusted blood hematocrit was predicted to decrease by 0.03% (95% CI: 0.01, 0.05) per 37 μg/g increase (mean of first and fourth quartiles) in patella bone Pb. The Makino et al. (1997) longitudinal study of pigment and vinyl chloride stabilizer manufacture workers (n = 1,573; blood Pb range 1–39 μg/dL) found a positive association between blood Pb concentration and hematocrit, and erythrocyte count. A simple linear regression model predicted an increase in hematocrit of 0.6 (typically 43) and an increase in erythrocyte count of $0.07 \times 10^6/mm^3$ (typically $4–7 \times 10^6/mm^3$) per 10 μg/dL increase in blood Pb concentration.

Studies that included subjects who had higher blood Pb concentrations (i.e., >40 μg/dL) have, in general, found negative associations between blood Pb concentration and hematocrit Gennart et al., 1992; Horiguchi et al., 1991; Poulos et al., 1986; Solliway et al., 1996), with two exceptions, Hsiao et al. (2001) and Osterode et al. (1999). Hsiao et al. (2001) conducted an 11-year retrospective longitudinal analysis of blood Pb concentration, hematocrit, and erythrocyte count in a group of battery manufacture workers (n = 30; mean blood Pb 30–60 μg/dL). A repeated measures regression analysis (generalized estimation equation) yielded

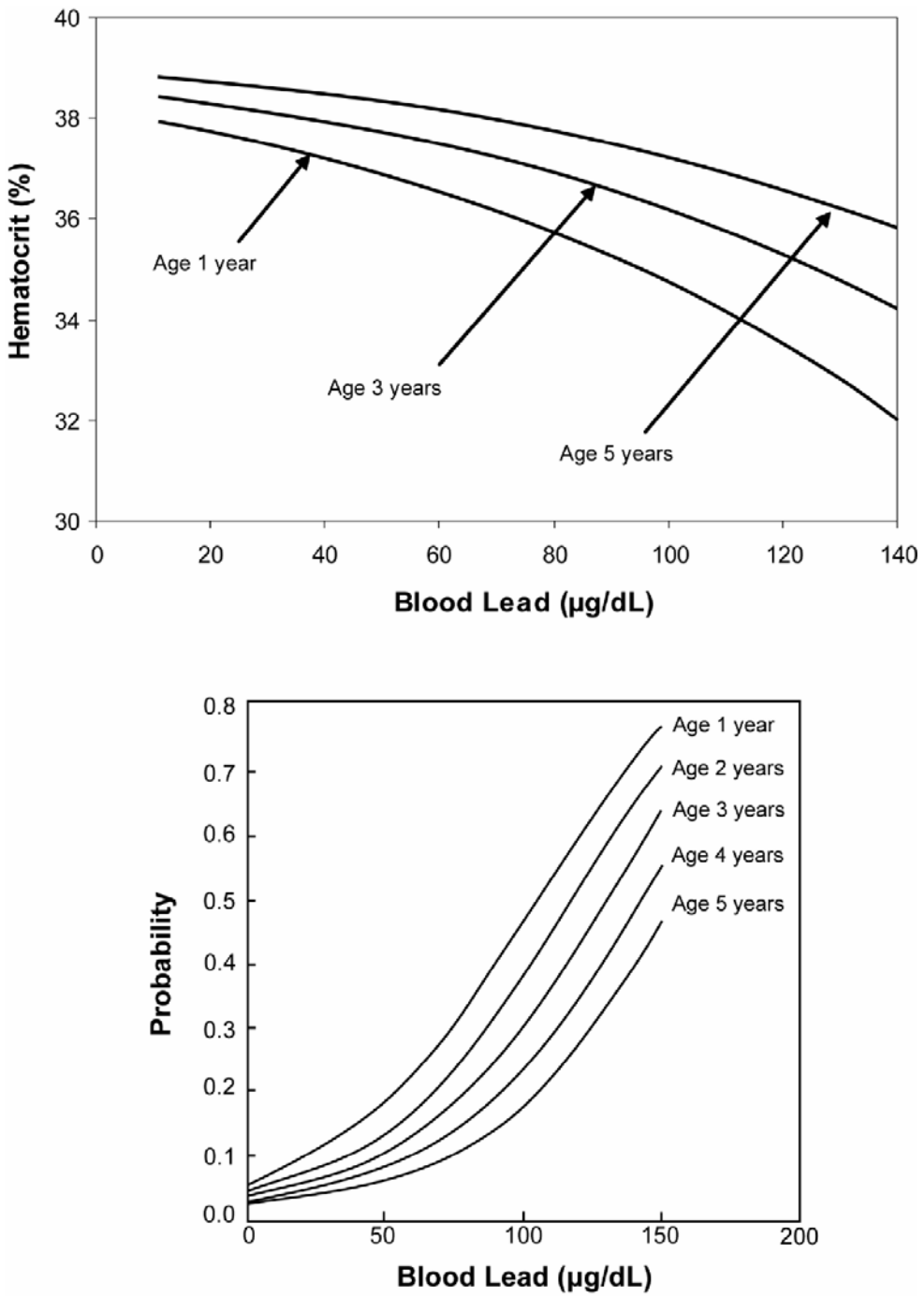

Figure 6-21. Relationship between blood lead and hematocrit in children. The top panel shows central tendency predictions based on a logistic regression model relating hematocrit and blood lead concentration, adjusted for age. The regression coefficients relating hematocrit and blood lead were (β = 0.0133 [SE 0.0041], p = 0.0005). The bottom panel shows corresponding concentration-response (hematocrit <35%) relationships.

Source: Schwartz et al. (1990).

a significant association between increasing blood Pb concentration and increasing hematocrit and erythrocyte count. Osterode et al. (1999) measured erythrocyte number and packed cell volume in a group of Pb workers (n = 20) and an age-matched reference group (n = 20). Mean blood Pb concentration was 45.5 µg/dL (range 16–91) in the Pb workers and 4.1 µg/dL (range 3–14) in the reference group. Mean erythrocyte number and packed cell volume in the Pb workers and reference group were not different.

6.9.2.4 Erythropoiesis

Several studies have found associations between Pb exposure and serum erythropoietin levels in children (Graziano et al., 2004; Liebelt et al., 1999) and adults (Graziano et al., 1991; Osterode et al., 1999; Romeo et al. 1996). A qualitative summary of outcomes from these studies are provided in Table 6-9.

Two studies have examined possible association between Pb exposure and serum erythropoietin levels in children. In the Kosovo prospective study (Factor-Litvak et al., 1998, 1999; Graziano et al., 2004) a significant association was evident between increasing blood Pb concentration (3–70 µg/dL) and increasing serum erythropoietin levels after adjustment for age and blood hemoglobin levels (Figure 6-22). The association weakened with age; it was significant at ages 4.5 and 6.5 years, but not at ages 9.5 or 12 years. A multivariate linear regression model predicted a 36% increase in serum erythropoietin per 10 µg/dL increase (3-13 µg/dL, hemoglobin 13 g/dL) in blood Pb at age 4.5 years and an 18% increase per 10 µg/dL at age 6.5 years. These outcomes suggest that erythropoiesis is stimulated in children in association with increasing blood Pb concentrations <40 µg/dL and in the absence of depressed blood hemoglobin levels.

A smaller cross-sectional study examined serum erythropoietin levels in a group of children (n = 89), 1 to 6 years of age (Liebelt et al., 1999). The blood Pb level range in the study group (2–84 µg/dL) was similar to that in the Graziano et al. (2004) study and, consistent with this study, Liebelt et al. (1999) found no association between blood Pb concentration and serum hemoglobin levels. However, in contrast to the Graziano et al. (2004) study, blood hemoglobin-adjusted serum erythropoietin levels decreased in association with an increase in blood Pb concentration (0.3 mIU/mL decrease per 10 µg/dL blood Pb increase). The Liebelt et al. (1999) study did not include age as a covariate in the regression model, which was shown in the Kosovo

Table 6-9. Summary of Results of Selected Studies of Associations Between Lead Exposure and Serum Erythropoietin

Study	Subjects	n[a]	Blood Lead (µg/dL) Mean (SD)	Blood Lead (µg/dL) Range	Serum Erythropoietin	Comment
Children						
Graziano et al. (2004)	Ages: 4.5–12 yr	311	6–9, 31–39[b]	3–70	+	Adjusted for age, blood hemoglobin
Liebelt et al. (1999)	Ages: 1–6 yr	86	18[c]	2–84	−	Adjusted for blood hemoglobin
Adults						
Graziano et al. (1990)	Pregnant women	48	NR	2–40	−	Stratified by blood hemoglobin
Osterode et al. (1999)	Male lead workers	40	45	16–91	−	Adjusted for blood packed cell volume
Romeo et al. (1996)	Male lead workers	141	30, 65[b,d]	30–92	−	No association with blood hemoglobin

−, decrease; +, increase; NR, not reported.

[a] Total number of subjects (including reference group)
[b] Range of means of low and higher exposure groups
[c] Median
[d] Reference group mean 10 µg/dL (range 3–20)

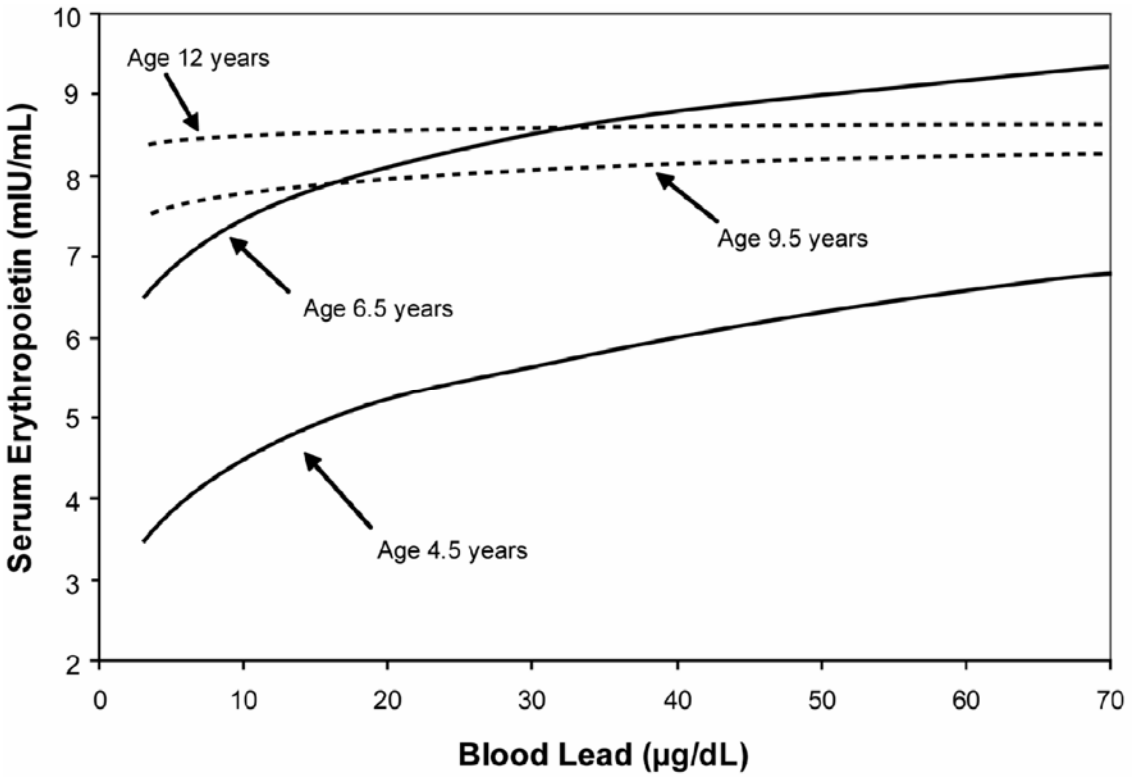

Figure 6-22. Relationship between blood lead and serum erythropoietin in children. Coefficients relating erythropoietin and blood lead were significant for ages 4.5 (β = 0.21 [95% CI: 0.13, 0.30], p < 0.0001) and 6.5 years (β = 0.12 [95% CI: 0.03, 0.20], p < 0.001).

Source: Graziano et al. (2004).

prospective study to be a significant covariable in blood Pb-serum erythropoietin relationship (Graziano et al., 2004); this may have contributed to the different outcome in the two studies. Liebelt et al. (1999) studied a convenience sample from a Pb/primary care clinic (rather than a prospectively selected cohort) that specifically excluded children who had symptoms of severe iron deficiency, or were taking iron supplements or other bone marrow suppressing drugs. Iron status of the children in the Graziano et al. (2004) study was not reported. However, serum ferritin levels in the mothers, at mid-pregnancy, was not indicative of iron deficiency (Graziano et al., 1990). Although the direction of the outcome measure was different in the two studies, both studies (Graziano et al., 2004; Liebelt et al., 1999) found evidence for an effect of Pb exposure on serum erythropoietin levels in the absence of significant Pb-associated changes in blood hemoglobin levels.

Three studies have found associations between Pb exposure and changes in erythropoiesis biomarkers in adults. As part of the Kosovo prospective study, serum erythropoietin was measured at mid-pregnancy and at term in a subset of women enrolled in the study (Graziano et al., 1991). The high- and low-Pb cohorts were constructed from the six highest and lowest mid-pregnancy blood Pb concentrations, within each of four blood hemoglobin strata, ranging from 9.0 to 12.9 g/dL. Mean blood Pb concentrations in the strata ranged from 17 to 39 μg/dL in the high-Pb group and 2.4 to 3.6 μg/dL in the low Pb group. Serum erythropoietin levels significantly decreased in association with increasing blood Pb concentration, independently of an effect of blood hemoglobin (Figure 6-23). Romeo et al. (1996) also found an association between increasing blood Pb concentration and decreasing serum erythropoietin, in the absence of discernable changes in blood hemoglobin levels, in a comparison of group male Pb workers (n = 28, blood Pb range 30–92 μg/dL) and a similar-aged reference group (n = 113, mean blood Pb 10 μg/dL [range 3–20]). Osterode et al. (1999) examined several measures of erythropoiesis in a group of Pb workers (n = 20, mean age 46 years) and in an age-matched reference group (n = 20). Mean blood Pb concentration was 45.5 μg/dL (range 16–91) in the Pb workers and 4.1 μg/dL (range 3–14) in the reference group. Mean blood hemoglobin levels in the Pb worker and reference groups were not different. Lead workers with blood Pb concentrations ≥60 μg/dL had significantly lower circulating erythrocyte progenitor cells than the reference group. Also, erythrocyte progenitor cell number was significantly negatively correlated with blood Pb and urine Pb concentrations. Serum erythropoietin levels increased exponentially with decreasing packed blood cell volume in the reference group, but not in the Pb workers (i.e., serum erythropoietin level was not significantly correlated with packed cell volume in the Pb workers). Thus, unlike the reference group (blood Pb concentration ≤14 μg/dL), Pb workers appeared to have a suppressed erythropoietin response to declining blood cell volume.

Collectively, the above studies suggest that Pb exposure depresses serum erythropoietin levels, in the absence of significant depression in blood hemoglobin levels. Lead-induced nephrotoxicity may contribute to a suppression of erythropoietin levels in Pb-exposed individuals. Although this cannot be entirely ruled out in these studies, both the Romeo et al. (1996) and Osterode et al. (1999) studies excluded people who had a history of hematological or kidney disease. Nevertheless, renal nephrotoxicity, including proximal tubular nephropathy,

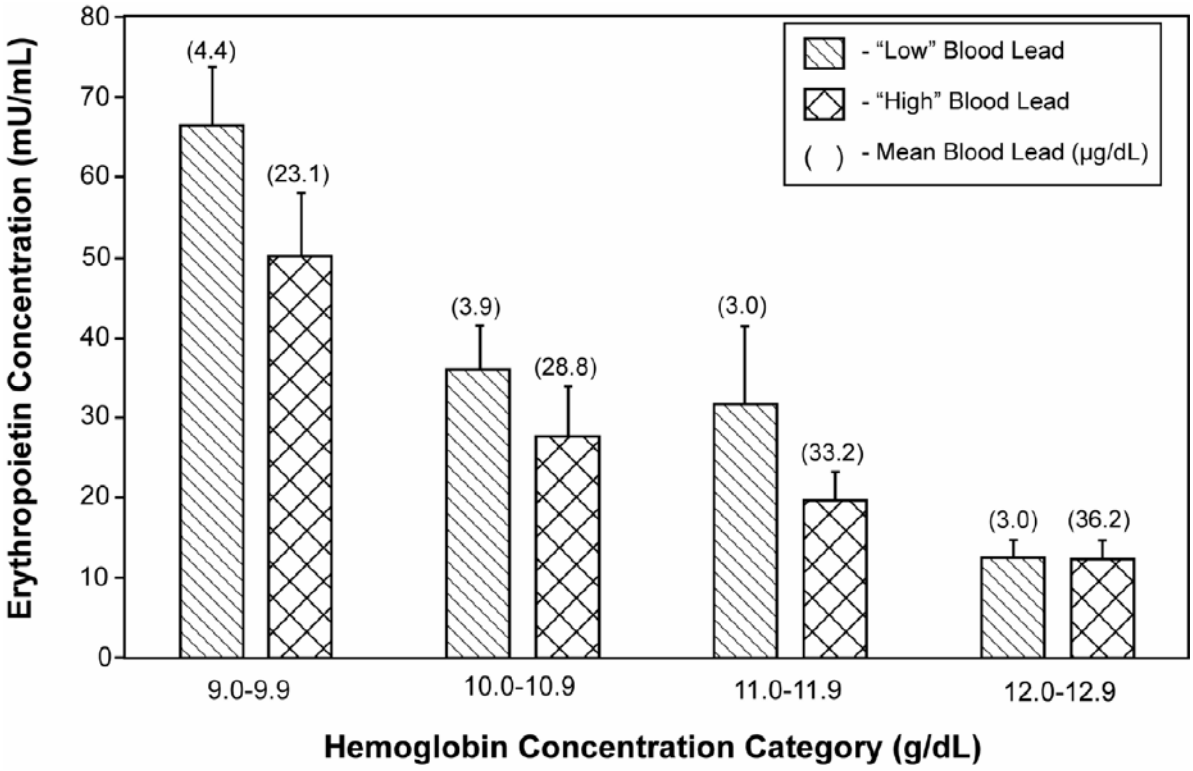

Figure 6-23. Association between blood lead concentration and serum erythropoietin in pregnant women. Shown are combined data for mid-pregnancy and delivery. Each bar represents the mean (±SD) of 12 subjects. ANOVA of the data at mid-pregnancy and at delivery showed blood lead effects (p = 0.049, p = 0.055, respectively) and blood hemoglobin effects (p = 0.0001, p = 0.009, respectively), with no significant interaction between the two variables.

Source: Graziano et al. (1991).

could have been a confounder in these studies which included subjects with blood Pb levels >40 µg/dL.

6.9.2.5 Other Effects on Erythrocyte Metabolism and Physiology

6.9.2.5.1 Erythrocyte Nucleotide Metabolism

Lead inhibits erythrocyte pyrymidine-5′nucleotidase (P5N) and adenine dinucleotide synthetase (NADS). Inhibition of P5N leads to the accumulation of pyrimidine nucleotides in the erythrocyte and hemolysis. Associations between increasing blood Pb concentration and decreasing blood P5N and NADS activity have been observed in studies of Pb workers (Kim

et al. 2002; Mohammed-Brahim et al., 1985; Morita et al., 1997). Mean blood Pb levels in these study groups were ≥35 µg/dL and ranged up to 80 µg/dL. Inhibition of P5N has also been observed in children whose blood Pb concentrations were >30 µg/dL (Angle and McIntire, 1978; Angle et al., 1982; summarized in the 1986 Lead AQCD).

6.9.2.5.2 Erythrocyte Deformability

Horiguchi et al. (1991) compared the deformability of erythrocytes collected from adult male secondary Pb refinery workers (n = 17, age range 24 to 58 years) with a reference group of male subjects (n = 13, age range 22 to 44 years). Erythrocyte deformability was assessed as microfilterability of erythrocytes under a negative (−10 cm H_2O) pressure head. Erythrocytes from the Pb workers showed significantly lower deformability compared to the reference group. The mean blood Pb concentration in the Pb workers was 53.5 µg/dL (SD 16.1); blood Pb values for the reference group were not reported.

6.9.2.5.3 Erythrocyte Membrane Transport

Hajem et al. (1990) measured erythrocyte membrane activities of Na^+-K^+-ATPase, Na^+-K^+-cotransport, Na^+-Li^+-antiport, and passive Na^+ and K^+ permeability in erythrocytes collected from adult males (n = 122; geometric mean blood Pb 16 µg/dL, range 8.0–33.0; geometric mean hair Pb 5.3 µg/g, range 0.9–60). Na^+-K^+-cotransport activity was negatively correlated with blood Pb concentration, but not with hair Pb. Na^+-K^+-ATPase activity was negatively correlated with hair Pb, but not with blood Pb.

6.9.3 Effects of Lead on the Endocrine System

6.9.3.1 Summary of Key Findings of the Effects of Lead on the Endocrine System from the 1986 Lead AQCD

The 1986 Lead AQCD concluded that various endocrine processes may be affected by Pb at relatively high exposure levels. These included effects on thyroid hormone levels (e.g., Refowitz, 1984; Robins et al., 1983), effects on male sex hormone levels (e.g., Braunstein et al., 1978), and impairment of the production of 1,25-dihydroxy vitamin D (1,25-$(OH)_2D_3$) (e.g., Rosen et al., 1980). Effects on these endocrine systems were concluded to be apparent only at blood Pb concentrations exceeding 30–40 µg/dL. The 1986 Lead AQCD concluded that studies

from which the effects of Pb on reproductive hormones in females could be assessed were lacking.

More recent epidemiologic studies have examined possible associations between Pb exposure (as reflected by blood and/or bone Pb levels) and various biomarkers of endocrine function, including the thyroid, male reproductive, and calcitropic endocrine systems. These studies have examined endocrine outcomes at lower blood Pb ranges and in the absence of overt clinical Pb toxicity, and have more rigorously attempted to control for confounding factors. Evidence for Pb effects on these systems, in association with blood Pb concentrations <30–40 μg/dL, remains absent. The strongest study designs have yielded no associations, or weak associations, between Pb exposure and thyroid hormone status (Erfurth et al., 2001; Schumacher et al., 1998; Tuppurainen et al., 1988; Zheng et al., 2001). Similarly, studies of the male reproductive system that attempted to control for confounding effects of age, have yielded mixed outcomes (Alexander et al., 1996a, 1998; Erfurth et al., 2001; Gustafson et al., 1989; McGregor and Mason, 1990; Ng et al., 1991). Results of a more recent epidemiologic study of the calcitropic endocrine system in children suggest that associations between serum vitamin D status and blood Pb may not be present in calcium-replete children who have average lifetime blood Pb concentrations <25 μg/dL (Koo et al., 1991). In adults, exposures to Pb that result in blood Pb concentrations >40–60 μg/dL may increase, rather than decrease, circulating levels of 1,25-$(OH)_2D_3$ and PTH (Kristal-Boneh et al., 1999; Mason et al., 1990), possibly as a compensatory response to increased urinary calcium losses secondary to impaired kidney function. Details regarding the design of these studies and outcomes are presented in Annex Tables AX6-9.5 and AX6-9.6. Outcomes of the most pertinent studies are summarized below.

6.9.3.2 Thyroid Endocrine Function

Several studies have examined possible associations between Pb exposure and thyroid hormone status. Most of these have been studies of occupational exposures. The results of these studies have been mixed; some studies have found significant associations with Pb exposure (e.g., blood Pb concentration), but most studies have found none or relatively weak associations. In studies that have controlled for the effects of age, outcomes also have been mixed, with the strongest study designs finding none or weak associations between Pb biomarkers and thyroid hormone status (Erfurth et al., 2001; Schumacher et al., 1998; Tuppurainen et al., 1988; Zheng

et al., 2001). The strength of the association and, possibly, the direction of the effect (i.e., increase or decrease in hormone levels) may change with exposure duration or level (Robins et al., 1983; Tuppurainen et al., 1988). The overall picture that emerges is that those studies that have included subjects having blood Pb levels >100 μg/dL have found depression of serum T3 and/or T4 levels, without a detectable increase in serum TSH. However, studies in which the blood Pb distribution was dominated by levels well below 100 μg/dL, have found either no effects or subclinical increases in serum T3, T4, with no change in TSH levels. Outcomes from the most pertinent studies are summarized qualitatively in Table 6-10 and are described in greater detail below.

Siegel et al. (1989) measured serum total thyroxine (TT4) and free thyroxine (FT4) in children ages 11 months to 7 years (n = 68) who were outpatients at a clinical care facility. Mean blood Pb concentration in the study group was 25 μg/dL (range 2–77). In a simple (univariate) linear regression analysis, hormone levels were not significantly associated with blood Pb concentration.

Zheng et al. (2001) measured concentrations of TT4 and transthyretin (TTR) in serum and cerebral spinal fluid (CSF) of adult hospital patients (n = 82) admitted for evaluation of CSF clinical chemistry (e.g., for head wounds, tumors, neurological symptoms). Mean blood Pb concentration was 14.9 μg/dL (SD 8.3). Age-adjusted serum TT4 and TTR, and CSF TT4 were not significantly associated with blood Pb concentration; however, increasing CSF Pb level was associated with decreasing CSF TTR levels ($r = -0.30$, $p = 0.023$).

Possible associations between Pb exposure and thyroid hormone status have been examined in several studies of Pb workers (Dursun and Tutus, 1999; Erfurth et al., 2001; Gennart et al., 1992; Gustafson et al., 1989; Horiguchi et al., 1987; Löpez et al., 2000; Refowitz, 1984; Robins et al., 1983; Schumacher et al., 1998; Singh et al., 2000; Tuppurainen et al., 1988). Of these, six warrant particular attention because the design and/or analysis attempted to control for effects of age (Erfurth et al., 2001; Dursun and Tutus, 1999; Gustafson et al., 1989; Schumacher et al., 1998; Tuppurainen et al., 1988; Robins et al., 1983). Outcomes of these studies are summarized in Table 6-10. The largest studies were Erfurth et al. (2001), Schumacher et al. (1998), and Tuppurainen et al. (1988).

Erfurth et al. (2001) was a cross-sectional study of secondary smelter workers (n = 62) and a reference group of metal (not Pb) workers (n = 26). Excluded from the study were

Table 6-10. Summary of Results of Selected Studies of Associations Between Lead Exposure and Thyroid Hormone Levels

Study	Subjects	n[a]	Blood Lead (µg/dL)		T3	T4	TSH
			Mean (SD)	Range			
Children							
Siegel et al. (1989)	Children, 11 mo–7 yrs	68	25	2–77	NR	o	NR
Adults							
Dursun and Tutus (1999)	Metal powder manufacture workers	57	17.1 (9.0)	1–36	+	+	o/o[b]
Erfurth et al. (2001)	Secondary smelter workers	88	31.1[c]	4–93	o	o	o
Gustafson et al. (1989)	Secondary smelter workers	42	39.4 (2.1)	NR	o	+	o
Robins et al. (1983)	Brass foundry workers	47	NR	16–127	NR	−	NR
Schumacher et al. (1998)	Primary smelter workers	151	24.1	<15 to ≥40%	o	o	o
Tuppurainen et al. (1988)	Battery manufacture workers	176	55.9 (23.8)	5–134	−	−	o
Zheng et al. (2001)	General population	82	14.9 (8.3)	NR	NR	o	NR

−, decrease; +, increase; o, no effect; NR, not reported; T3, triiodothyronine; T4, thyroxine; TSH, thyroid stimulating hormone.

[a] Total number of subjects (including reference group)
[b] Basal/thyroid releasing hormone-stimulated
[c] Median

individuals with ongoing thyroid disease or who were taking thyroid hormone supplements or other drugs that would interfere with thyroid hormone levels (e.g., beta-blockers). Median blood Pb concentration in the Pb workers was 31 µg/dL (range 8–93). Age-adjusted basal serum levels of FT3, FT4, and TSH were not associated with blood, urine, or finger bone Pb levels. Thyroid releasing hormone (TRH)-induced TSH secretion (area under serum TSH concentration-time curve) was measured in an age-matched subset of the study group (9 Pb workers and 11 reference subjects) and was not significantly different in the two groups. The Schumacher et al. (1998) study measured serum FT4, TT4, and TSH levels in a group of male workers (n = 151) at the Trail British Columbia smelter complex. Excluded from the study were individuals who had ongoing clinical thyroid disease. Mean blood Pb concentration in the study group was 24 µg/dL (15% >40 µg/dL). Covariate-adjusted (age, alcohol consumption) hormone levels were not significantly associated with current blood Pb concentration or 10-year average blood Pb concentrations. Prevalence of abnormal hormone values was also unrelated to blood Pb concentration.

Tuppurainen et al. (1988) measured serum total triiodothyronine (TT3), FT4, TT4, and TSH levels in a group of male battery manufacture workers (n = 176). Mean blood Pb concentration was 56 µg/dL (range 14–134). Although, hormone levels were not significantly associated with blood Pb concentrations, increasing exposure (i.e., employment) duration was significantly associated with decreasing FT4 ($r^2 = 0.071$, $p = 0.001$) and TT4 ($r^2 = 0.059$, $p = 0.021$) levels. The r^2 was not improved by including age or blood Pb as covariables. Strength of the association was greater when the analysis was restricted to workers who had an exposure duration >7.6 years (FT4: $r^2 = 0.33$, $p < 0.002$; TT4: $r^2 = 0.21$, $p < 0.001$). Consistent with the results of the Tuppurainen et al. (1988) study, Robins et al. (1983) found a significant association between increasing blood Pb concentration and decreasing FT4 ($r^2 = 0.085$, $p = 0.048$) in a group of brass foundry workers (n = 47). The blood Pb range in the subjects was 16–127 µg/dL. When stratified by race (Black, White) the association was significant in the Black stratum ($r^2 = 0.21$, $p = 0.03$), but not in the White stratum ($r^2 = 0.05$, $p = 0.27$). The strength of association was not changed by including age in the regression model. Both the Robins et al. (1983) and Tuppurainen et al. (1988) included subjects with blood Pb levels >100 µg/dL.

Blood Pb concentrations were lower in the Dursun and Tutus (1999) and Gustafson et al. (1989) studies than in the above studies, and both studies found significant associations between Pb exposure and increasing serum TT4 levels. Dursun and Tutus (1999) measured serum FT3, TT3, FT4, TT4, and TSH in a group of metal powder manufacture workers (n = 27) and a reference group (n = 30). Mean blood Pb concentration in the workers was 17 µg/dL (range 9-36). A linear regression model that included age, blood Pb concentration, and exposure duration, indicated a significant association between increasing exposure duration and increasing serum TT4 levels ($r^2 = 0.3$, $p = 0.03$). The Gustafson et al. (1989) study examined a group of male secondary smelter workers (n = 21) and reference subjects, individually matched to the Pb workers by age, sex, and work shift. Mean blood Pb concentration in the workers was 39 µg/dL (SD 2). Serum TT4 levels were significantly higher ($p < 0.02$) in the Pb workers compared to the reference group. The difference strengthened when the analysis was restricted to the age range <40 years ($p = 0.01$).

6.9.3.3 Reproductive Endocrine Function

6.9.3.3.1 Male Reproductive Endocrine Function

Low testosterone (TES) levels, blunted sex hormone secretion in response to gonadotropin releasing hormone (GnRH), and defects in spermatogenesis have been observed in humans exhibiting clinical neurological symptoms of Pb poisoning (Braunstein et al., 1978; Cullen et al., 1984). However, the effects of lower exposure levels on reproductive endocrine status are less clear. Possible associations between Pb exposure and changes in male reproductive hormone levels have been examined in studies of Pb workers. Of these, five studies attempted to control for effects of age, an important determinant of testosterone levels (Alexander et al., 1998; Erfurth et al., 2001; Gustafson et al., 1989; McGregor and Mason, 1990; Ng et al., 1991). The outcomes from these studies are qualitatively summarized in Table 6-11. Blood Pb ranges in the latter studies were similar (4 to 90 µg/dL), yet outcomes were mixed, with observations of no change (Erfurth et al., 2001; Gustafson et al., 1989; McGregor and Mason, 1990) or subclinical decrease (Alexander et al., 1996a, 1998; Ng et al., 1991) in serum testosterone (TES) in association with Pb exposure. Mixed effects were observed for the effect of Pb exposure on serum follicle stimulating hormone (FSH) and luteinizing hormone (LH),

Table 6-11. Summary of Results of Selected Studies of Associations Between Lead Exposure and Male Sex Hormone Levels in Adults

Study	Subjects	n[a]	Blood Lead (µg/dL)		FSH	LH	PRL	TES
			Mean (SD)	Range				
Alexander et al. (1996a, 1998)	Primary smelter workers	152	NR	5–58	o	o	NR	–[b]
Erfurth et al. (2001)	Secondary smelter workers	88	31.1[c]	4–93	o/-[d,e]	o/o[d]	o/o[d]	o[d]
Gustafson et al. (1989)	Secondary smelter workers	42	39.4 (2.1)	NR	–	–	o	o
McGregor and Mason (1990)	Lead workers	176	NR	17–77	+	+	NR	o
Ng et al. (1991)	Battery manufacture workers	171	35 (13)	10–72	+	+	o	–

–, decrease; +, increase; o, no effect; NR, not reported; FSH, follicle stimulating hormone; LH, luteinizing hormone; PRL, prolactin; TES, testosterone.

[a] Total number of subjects (including reference group)
[b] In association with increasing semen lead levels, not with blood lead
[c] Median
[d] Basal/gonadotropin releasing hormone-stimulated
[e] Effect was evident in comparison between groups, but not in multivariate regression that adjusted for age

increases (McGregor and Mason, 1990; Ng et al., 1991), decreases (Gustafson et al., 1989), and with no change (Alexander et al., 1996a, 1998; Erfurth et al., 2001) in hormone levels observed.

The inconsistency in the direction of effects on TES and the two androgen regulating pituitary hormones, FSH and LH, is particularly noteworthy, and suggest the possibility of multiple effect of Pb on the hypothalamic-pituitary-gonad axis, consistent with observations that have been made in some experimental animal studies. Erfurth et al. (2001) observed a suppressed FSH response to GnRH in a group of Pb workers compared to an age matched reference group; however, the magnitude of the response was not significantly associated with Pb exposure indices in a multivariate regression analysis that accounted for age. In rats, Pb exposure can suppress serum testosterone levels in the absence of a change in circulating levels of GnRH or LH, even though levels of GnRH mRNA increase in the hypothalamus (Klein et al., 1994; Ronis et al., 1996; Sokol et al. 2002). Thus, changes in GnRH production, at the molecular level, do not necessarily translate to changes in hormone levels. This may be the result of Pb inhibition of release of GnRH for nerve terminals in the median eminence (Bratton et al., 1994; Sokol, 1987; Sokol et al., 1998, 2002).

Alexander et al. (1996a, 1998) examined serum FSH, LH, and TES in males (n = 152) who worked at the Trail British Columbia smelter complex. Covariate-adjusted hormone levels and prevalence of clinically abnormal values were unrelated ($p \geq 0.05$) to blood Pb level (range 5–58 µg/dL); however, increasing semen Pb concentration (range 0.3–17 µg/dL) was significantly associated with decreasing semen testosterone levels ($p = 0.004$). Erfurth et al. (2001) measured serum TES, sex hormone binding globulin (SHBG), and GnRH-stimulated changes in serum FS, LH, and PRL in male secondary smelter workers (n = 62) and in a reference group (n = 26). Mean blood Pb in the Pb workers was 31 µg/dL (range 8–93). Age-adjusted basal hormone levels were unrelated to blood, plasma, or urine Pb concentrations. In an age-matched subset of the cohorts (n = 9 Pb workers, n = 11 reference), median GnRH-stimulated serum FSH was significantly lower in Pb workers than in the reference group; however, GnRH-stimulated LH, FSH, and PRL were not significantly associated with any of the Pb measures in a multivariate regression analysis. Gustafson et al. (1989) measured serum FSH, LH, and TES (total and free) in a group of male secondary smelter workers (n = 21) and in a group of reference subjects individually matched to the Pb workers by age, sex, and work shift. Mean blood Pb concentrations were 39 µg/dL (SD 2) in the Pb workers and 5.0 µg/dL (SD 0.2)

in the reference group. Serum FSH levels were significantly lower ($p = 0.009$) in Pb workers compared to reference group. When the analysis was restricted to the age range <40 years, Pb workers had significantly lower FSH and LH compared to the reference group. McGregor and Mason (1990) measured serum FSH, LH, TES, and SHBG in a group of male Pb workers ($n = 90$) and in a reference group ($n = 86$). Blood Pb range in the Pb workers was 17–77 μg/dL; blood Pb concentrations in the reference subjects were <12 μg/dL. Prevalences of abnormal hormone levels in the Pb workers and reference group were not different; however, age-adjusted serum FSH was significantly higher in Pb workers compared to reference group and increasing FSH levels were significantly associated with increasing blood Pb concentrations. Increasing serum LH was significantly associated with increasing exposure duration but not with blood Pb concentration or age. Serum TES or SHBG levels were unrelated to blood Pb concentration or exposure duration. Ng et al. (1991) measured serum FSH, LH, PRL, and TES in a group of male battery manufacture workers ($n = 122$) and a reference group ($n = 49$). Mean blood Pb levels were 35 μg/dL (range 10–77) in the Pb workers and 8 μg/dL (range 3-15) in the reference group. When cohorts were stratified by age, serum FSH and LH levels were significantly higher in Pb workers <40 years of age compared to corresponding age stratum of the reference group; serum TES was significantly lower in Pb workers ≥40 years of age, compared to the same age stratum in the reference group. Covariate-adjusted (age, tobacco smoking) serum TES levels were significantly lower in Pb workers in the 10-year exposure duration stratum, compared to the reference group. Covariate-adjusted serum FSH and LH were significantly higher in Pb workers in the <10-year exposure duration stratum, compared to the reference group.

6.9.3.3.2 Female Reproductive Endocrine Function

Although delays in sexual maturation in humans have been associated with increases in blood Pb concentrations (Selevan et al., 2003; Wu et al., 2003b), and Pb has been shown to alter levels of female sex hormones and the menstrual cycle in nonhuman primates (Foster, 1992; Franks et al., 1989; Laughlin et al., 1987), epidemiologic studies of interactions between Pb exposure and reproductive endocrinology in females have not been reported. Lead introduced into cultures of human ovarian granulosa cells suppresses progesterone production (Paksy et al., 2001) and suppresses expression of aromatase and estrogen receptor β (Taupeau et al., 2003).

6.9.3.4 Pituitary and Adrenal Endocrine Function

Several studies of possible associations between Pb exposure and levels of pituitary hormones that regulate production and secretion of thyroid hormones (see Section 6.9.3.2) and reproductive hormones (see Section 6.9.3.3) have been reported. In addition to the above studies, Gustafson et al. (1989) found that serum cortisol levels were lower in a group of male secondary smelter workers (n = 21) compared to a reference group individually matched to the Pb workers by age, sex, and work shift. Mean blood Pb concentrations were 39 μg/dL (SD 2) in the workers and 5.0 μg/dL (SD 0.2) in the reference group. Campbell et al. (1985) measured various biomarkers of status of the renin-angiotensin-aldosterone system in male welders (n = 5) and reference subjects (n = 8). Mean blood Pb concentration was 35 μg/dL (range 8-62 μg/dL). Significant positive correlations were observed between blood Pb concentration and plasma aldosterone ($r = 0.53$, $p < 0.002$), which may have been, at least in part, secondary to a Pb effect on plasma renin activity ($r = -0.76$, $p < 0.001$) and angiotensin I levels ($r = 0.68$, $p < 0.002$). Saenger et al. (1984) found lower urinary levels of 6-β-OH-cortisol, but not cortisol, in children who had elevated urinary Pb in an EDTA provocation test (>500 μg/24 h), compared to children who did not have elevated urinary Pb levels, or whose blood Pb levels were <30 μg/dL. The change in urinary excretion of 6-β-OH-cortisol in the absence of a change in cortisol levels may reflect an effect of Pb on liver cytochrome P450 activity, rather than an effect on the adrenal gland (see Section 6.9.4).

6.9.3.5 Calcitropic Endocrine Function

Children exposed to relatively high level of Pb >30 μg/dL may exhibit depressed levels of circulating 1,25-$(OH)_2D_3$ (Mahaffey et al., 1982; Rosen et al., 1980). These effects were not detected in a study of calcium-replete children with average lifetime blood Pb levels below 25 μg/dL (Koo et al., 1991). In adults, Pb exposures that result in blood Pb concentrations >40-60 μg/dL may increase, rather than decrease, circulating levels of 1,25-$(OH)_2D_3$ and PTH. These studies also are summarized in Annex Tables AX6-9.5 and AX6-9.6. Outcomes from the more pertinent studies are qualitatively summarized in Table 6-12 and are discussed in greater detail below.

Epidemiologic studies of possible associations between Pb exposure and vitamin D status in children have yielded mixed results. Mahaffey et al. (1982) and Rosen et al. (1980) observed

Table 6-12. Summary of Results of Selected Studies of Associations Between Lead Exposure and Calcitropic Hormones

Study	Subjects	n[a]	Blood Lead (μg/dL)		PTH	CAL	1,25D	25D
			Mean (SD)	Range				
Children								
Koo et al. (1991)	Ages: 21, 27, 33 mo	105	9.7	5–24	o	o	o	o
Mahaffey et al. (1982)	Ages: 1–16 yr	177	NR	12–120	o	o	−	o
Rosen et al. (1980)	Ages: 1–5 yr	45	18, 47, 74[b]	10–120	+	o	−	−
Adults								
Chalkley et al. (1998)	Smelter workers[c]	19	47	21–76	NR	NR	+[c]	o
Kristal-Boneh et al. (1998)	Battery manufacture workers	140	43	1–77	+	NR	+	NR
Mason et al. (1990)	Lead workers	138	NR	15–95	o	NR	+	NR

−, decrease; +, increase; o, no effect; NR, not reported; PTH, parathyroid hormone; CAL, calcitonin; 1,25D, 1,25-dihydroxyvitamin D; 25D, 25-hydroxyvitamin D.

[a] Total number of subjects (including reference group)
[b] Group means: low, moderate, high
[c] Cadmium, lead, zinc smelter workers, effect on 1,24D in association with high blood cadmium and lead and high urinary cadmium

lower 1,25-$(OH)_2D_3$ in association with increasing blood Pb concentration. Koo et al. (1991) found no association between 1,25-$(OH)_2D_3$ and blood Pb concentration. The Koo et al. (1991) study was a longitudinal analysis of a subset of a prospective study of pregnancy outcomes. Serum calcium, magnesium, phosphorus, PTH, CAL, 25-OH-D_3, 1,25-$(OH)_2D_3$, and bone mineral content were measured in children (n = 105) at ages 21, 27, and 33 months. Mean lifetime average blood Pb concentrations (based on quarterly assessments) was 9.7 µg/dL (range 4.8–23.6). The range of highest values observed was 6 to 63 µg/dL. A structural equation model was developed that initially considered age, sex, race, sampling season, and dietary intake of calcium, phosphorus, and vitamin D as covariables; the final model retained age, sex, race, and sampling season. Decreasing blood Pb (ln-transformed) was significantly associated with covariate-adjusted decreasing serum phosphorus. No other covariate-adjusted outcomes were significantly associated with blood Pb. The distribution of dietary calcium intakes was 4% for ≤600 mg/day, 55% for 600–1200 mg/day, and 41% for >1200 mg/day. Intakes of phosphorous were similar, suggesting that the subjects were nutritionally replete with respect to these two nutrients.

The different outcomes in Koo et al. (1991) compared to the Mahaffey et al. (1982) and Rosen et al. (1980) studies may reflect, in part, the lower blood Pb range in the subjects in Koo et al. (1991) (range of lifetime average 5–24 µg/dL, range of observed highest values 6–63 µg/dL) compared to the Mahaffey et al. (1982) and Rosen et al. (1980) studies (10–120 µg/dL). Subjects in the Koo et al. (1991) study also had higher calcium intakes (4% with ≤600 mg/day, 43% with >1200 mg/day) than in the Rosen et al. (1980) study (mean 580 mg/day [SE 15] in high blood Pb group). Calcium intake (and/or related nutritional factors) may also have been an uncontrolled confounder in the Rosen et al. (1980) study, as higher blood Pb concentration appeared to be associated with lower calcium intakes (Sorrell et al., 1977). Mahaffey et al. (1982) did not report calcium intakes. Thus, the effect of Pb exposure on vitamin D status may be more pronounced at higher blood Pb concentrations (i.e., >60 µg/dL) and in combination with lower intakes of calcium (or other nutritional limitations).

Studies of Pb workers have found evidence for higher serum levels of 1,25-$(OH)_2D_3$ and PTH in association with increasing blood Pb concentration (Chalkley et al., 1998; Kristal-Boneh et al., 1998; Mason et al., 1990). The Chalkley et al. (1998) study was a small study (n = 19) of subjects exposed to both Cd and Pb, and effects of Pb and Cd on 1,25-$(OH)_2D_3$ could not be

isolated. The Kristal-Boneh et al. (1998) and Mason et al. (1990) studies included larger samples of subjects whose exposure was primarily, but not exclusively, to Pb. Attempts were made to control for effects of age and, in the Kristal-Boneh et al. (1998) study, other potential covariables. Kristal-Boneh et al. (1998) measured serum calcium, magnesium, phosphorus, PTH, 25-OH-D_3, and 1,25-$(OH)_2D_3$ in a group of male battery manufacture workers (n = 56) and a reference group (n = 90). Mean blood Pb concentrations were 43 μg/dL (SD 14, range 1–77) in the Pb worker group and 4.5 μg/dL (SD 2.6, range 1.4–19) in the reference group. Serum 1,25-$(OH)_2D_3$ and PTH, but not 25-OH-D_3, were significantly higher in Pb workers compared to the reference group. Increasing blood Pb concentration (ln-transformed) was significantly associated with covariate-adjusted increasing serum PTH and 1,25-$(OH)_2D_3$ levels. No effects on serum calcium were apparent. Occupational Pb exposure was also significantly associated with increasing PTH and 1,25-$(OH)_2D_3$ level. Covariates retained in the multivariate model were age, alcohol consumption, smoking; calcium intake, magnesium intake, and calorie intake. Mason et al. (1990) measured serum calcium, phosphate, PTH, and 1,25-$(OH)_2D_3$ in male Pb workers (n = 63) and in a reference group (n = 75) and found significantly higher prevalence of elevated 1,25-$(OH)_2D_3$ (defined as >2 SD higher than reference mean) in Pb workers (13%) compared to the reference group (1.3%). Serum levels of 1,25-$(OH)_2D_3$ were also significantly higher in Pb workers compared to the reference group. After stratification of the Pb workers into exposure categories (high exposure: blood Pb ≥40 μg/dL and bone Pb ≥40 μg/g; low exposure: blood Pb ≤40 μg/dL and bone Pb ≤40 μg/g), serum 1,25-$(OH)_2D_3$ levels were significantly higher in the high Pb group. Serum calcium levels were not different in the two groups. Increasing blood Pb was significantly associated with increasing 1,25-$(OH)_2D_3$ levels (r^2 = 0.206; with age and bone Pb included, r^2 = 0.218). After excluding 12 subjects whose blood Pb concentrations >60 μg/dL, the regression coefficient was no longer significant (r^2 = 0.162, p = 0.26).

6.9.4 Effects of Lead on the Hepatic System

6.9.4.1 Summary of Key Findings of the Effects of Lead on the Hepatic System from the 1986 Lead AQCD

The 1986 Lead AQCD noted that effects of Pb on liver function in humans had not been extensively studied. Possible association between Pb exposures (blood Pb concentrations

>70 µg/dL) and nonspecific liver injury (i.e., increases in liver enzymes in serum) were noted based on studies of workers (e.g., Cooper et al., 1973; Hammond et al., 1980). Also noted was evidence for possible association of suppression of hepatic cytochrome P450 activity with high blood Pb concentrations (>70 µg/dL) (Meredith et al., 1977).

Few studies of hepatic effects of Pb on humans have been reported since the 1986 Lead AQCD. Studies of hepatic enzyme levels in serum suggest that liver injury may be present in Pb workers; however, associations specifically with Pb exposures are not evident (Al-Neamy et al., 2001; Hsiao et al., 2001). Studies of urinary metabolites of cytochrome P450 phenotypes CYP2A6 and CYP3A4 suggest possible associations between Pb exposure and suppression of hepatic enzyme activity. The effect on CYP2A6 activity was observed in children with high Pb burdens (i.e., blood Pb concentration >40 µg/dL, EDTA-provoked urinary Pb >500 µg/dL). The effect on CYP3A4 was observed in association with blood Pb ranges of ~30-112 µg/dL (based on reported serum Pb concentrations). These studies are summarized in Annex Table AX6-9.7 and the most pertinent findings are discussed below.

6.9.4.2 Nonspecific Hepatic Injury

Possible association between occupational Pb exposure and liver injury has been assessed from measurements of serum enzymes (Al-Neamy et al., 2001; Hsiao et al., 2001). Al-Neamy et al. (2001) found significantly higher serum activity of alkaline phosphatase (AP) and lactate dehydrogenase (LDH), both within clinically normal ranges, in a group (n = 100) of male Pb workers (e.g., gas pump attendants, garage workers, printing workers, construction workers), compared to an age-matched reference group (n = 100). Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transferase (γ-GT) were not different in the two groups. The mean blood Pb concentrations were 78 µg/dL (SD 43) in the Pb workers and 20 µg/dL (SD 12) in the reference group. Hsiao et al. (2001) found no association between blood Pb concentration and ALT activity, in a longitudinal study of a group of battery manufactory workers (n = 30). Mean blood Pb concentrations ranged from 60 µg/dL (~range 25–100) at the start of the study (1989) to 30 µg/dL (~range 10–60) in the final year of the study (1999).

6.9.4.3 Hepatic Cytochrome P450 Function

Studies conducted in animals have shown that Pb can decrease the activity of hepatic cytochrome P450 and its induction by various inducing agent, through a mechanism that, at least in part, involves a disruption of heme synthesis (see Section 5.10.1.1). Possible associations between Pb exposure and cytochrome P450 activity have been studied in children and adults (Saenger et al., 1984; Satarug et al., 2004). Although direct assay of hepatic cytochrome P450 levels is not feasible in epidemiological studies, changes in activities of P450 isozymes can be detected from measurements of urinary metabolites of P450 substrates. Urinary excretion of 6-β-hydroxycortisol (6-β-OH-cortisol) derives primarily from oxidation of cortisol through the hepatic cytochrome P450 phenotype CYP3A4. A lower urinary 6-β-OH-cortisol:cortisol ratio is indicative of possible suppression of hepatic CYP3A4 activity. Saenger et al. (1984) found significantly lower (~45% lower) urinary excretion of 6-β-OH-cortisol and lower urinary 6-β-OH-cortisol:cortisol ratio in 2 to 9 year-old children ($n = 26$) who qualified for chelation (EDTA-provoked urinary Pb >500 μg/24 h) than in children who did not qualify, and significantly lower than in an age-matched reference group. Urinary 6-β-OH-cortisol:cortisol ratio was significantly correlated with blood Pb ($r = -0.514$, $p < 0.001$), urinary Pb, and EDTA-provoked urinary Pb ($r = -0.593$, $p < 0.001$). Mean blood Pb concentrations were 46 μg/dL (range 33–60), prior to chelation, and 42 μg/dL (range 32–60) in the children who did not qualify for chelation.

Satarug et al. (2004) measured urinary excretion of 7-hydroxy-coumarin (7-OH-coumarin) following a single oral dose of coumarin to assess effects of Cd and Pb exposure on cytochrome P450 phenotype CYP2A6. The rationale for this approach is that 7-hydroxylation of coumarin occurs solely through the CYP2A6 pathway. Coumarin-induced urinary 7-OH-coumarin was measured in a group ($n = 118$) selected from the general population in Bangkok, Thailand. All subjects were nonsmokers. The study found a significant association between increasing urinary Pb and decreasing covariate-adjusted urinary 7-OH-coumarin in males, but not in females. Covariates retained included age and zinc excretion. A significant association, in opposite direction, was found between urinary Cd and urinary 7-OH-coumarin. Mean urinary Pb levels (blood Pb concentrations were not reported) were 1.3 μg/g creatinine (range 0.1–12) in males, and 2.4 μg/g creatinine (range 0.6–6.8) in females. Mean serum Pb concentrations were 4.2 μg/L (range 1–28) in males and 3.0 μg/L (range 1–12) in females. The range of 1 to 28 μg/L

serum would correspond to a blood Pb concentration range of ~30 to 112 µg/dL (U.S. Environmental Protection Agency, 2003). These results are consistent with observations of depressed excretion of metabolites of the CYP2A6 substrate, phenazone, in association with overt clinical Pb toxicity in Pb workers (Fischbein et al., 1977; Meredith et al., 1977).

6.9.5 Effects of Lead on the Gastrointestinal System

6.9.5.1 Summary of Key Findings on the Effects of Lead on the Gastrointestinal System from the 1986 Lead AQCD

The 1986 Lead AQCD described gastrointestinal colic (abdominal pain, constipation, intestinal paralysis) as a consistent early symptom of Pb poisoning in humans and noted that such GI symptoms may be present in association with blood Pb concentrations in the range of 30-80 µg/dL. The 1986 Lead AQCD concluded that information was insufficient to establish clear concentration (i.e., blood concentration)-response relationships in the general population in association with environmental exposure. Subsequent to the 1986 AQCD several studies of prevalence of symptoms of GI colic in Pb workers have been reported that provide evidence for symptoms in association with blood Pb levels >50–80 µg/dL (Awad el Karim et al., 1986; Holness and Nethercott, 1988; Lee et al., 2000; Matte et al., 1989). These studies are summarized in Annex Table AX6-9.8. Similar types of studies of children have not been reported.

6.9.5.2 Gastrointestinal Colic

Lee et al. (2000) collected data on symptoms (self-reported questionnaire) in male Pb workers (n = 95) who worked in secondary smelters, PVC-stabilizer manufacture facilities, or battery manufacture facilities. A logistic regression model was applied to prevalence data for GI symptoms (loss of appetite, constipation or diarrhea, abdominal pain). The covariate-adjusted odds ratio for symptoms, in association with blood Pb concentration (≥ versus < the group median, 45.7 µg/dL), was not significant (1.8, [95% CI: 0.7, 4.5]). The corresponding odds ratio for DMSA-provoked urinary Pb (≥versus <260.5 µg/4 h, the group median) was also not significant (1.1, [95% CI: 0.4, 2.5]). However, the odds ratio for neuromuscular symptoms in association with DMSA-provoked urinary Pb was significant (7.8, [95% CI: 2.8, 24.5]), suggesting that neuromuscular symptoms may occur in association with exposures insufficient to

result in detectable GI symptoms. Covariates retained in the final regression models were age, tobacco smoking, and alcohol consumption.

Three other studies have attempted to quantify associations between Pb exposure and GI symptoms in Pb workers (Awad el Karim et al., 1986; Holness and Nethercott, 1988; Matte et al., 1989). Holness and Nethercott (1988) found a significantly ($p < 0.05$) higher prevalence of symptoms in a group of demolition workers (n = 119) in association with a blood Pb range of 50 to 70 µg/dL (n = 87), 37% for abdominal cramps and 42% for constipation, or >70 µg/dL (n = 19) 77% for abdominal cramps and 62% for constipation compared to a group of workers in which the blood Pb values were <50 µg/dL (n = 13), prevalences of 8% and 6%. Awad el Karim et al. (1986) found higher prevalence of GI symptoms, for abdominal colic and constipation, respectively, in male battery manufacture workers, 41.3% for abdominal colic and 41.4% for constipation, compared to a reference group of workers, n = 40 prevalences of 7.5% and 10% for abdominal colic and constipation, respectively. The blood Pb ranges were 55 to 81 µg/dL in the Pb workers and 7 to 33 µg/dL in the reference group. Matte et al. (1989) did not find a significant difference in prevalence of GI symptoms (decreased appetite, nausea, abdominal pain) among a group of battery manufacture and repair workers (n = 63) when stratified by blood Pb concentration (<60 µg/dL, ≥60 µg/dL). The prevalence ratio (high/low blood Pb strata) for abdominal pain was 1.5 (95% CI: 0.5, 4.6).

In a small study of environmentally-exposed adults, Bercovitz and Laufer (1991) found that Pb levels in the dentine of patients with GI ulcers (n = 11), even long after recovery, were significantly higher (mean Pb 75.02 µg/g [SE 8.15]) than those in healthy subjects (mean Pb 25.62 µg/g [SE 10.15]). Ten of the 11 peptic ulcer patients had a higher Pb level than the healthy subjects. In these 10 patients, increased severity of the ulcer and longevity of suffering was associated with increased tooth Pb levels. The authors suggested that increased Pb absorption was associated with damage to the epithelial mucosal cells of the GI tract.

6.9.6 Effects of Lead on Bone and Teeth

6.9.6.1 Summary of Key Findings of the Effects of Lead on Bone and Teeth from the 1986 Lead AQCD

The 1986 Lead AQCD did not discuss the effects of Pb on bone and teeth. Since the 1986 AQCD, an additional development in Pb epidemiology has been studies which explored possible

associations between Pb exposure and risk of dental caries (Campbell et al., 2000; Dye et al., 2002; Gemmel et al., 2002; Moss et al., 1999). Also, a limited number of studies also examined the toxic effect of Pb on bone. These studies are summarized in Annex Table AX6-9.9.

6.9.6.2 Bone Toxicity

The number of papers dealing with direct toxicity of Pb on bone is limited. Most papers are reviews (Hu et al., 1991; Puzas, 2000; Puzas et al., 1992; Rabinowitz, 1991; Silbergeld, 1991; Silbergeld et al., 1993; Vig and Hu, 2000) or are based on cellular studies (e.g., Pounds et al., 1991) or laboratory animal evaluations.

Various authors have suggested that Pb is a potential risk factor for osteoporosis because of the pivotal role of the skeleton in Pb toxicokinetics (Goyer et al., 1994). Bone cells accumulate Pb actively and earlier ideas suggested that Pb was incorporated into the mineral matrix of the bone (Wittmers et al., 1988). However, in an in vivo iliac bone biopsy using laser microbeam mass analysis on a Pb-intoxicated adult female following chelation therapy, Flood et al. (1988) found the extracellular Pb was concentrated in the superficial 3 to 6 μm of the osteoid zone of bony trabeculae. Because Pb was absent from the deeper parts of the mineralized matrix, the authors suggested that Pb binds more strongly to the organic matrix than to bone mineral.

There is increasing evidence from cell culture experiments, animal studies, and from measurements in humans that Pb may exert detrimental effects on bone mineral metabolism. In humans, this evidence comes from several studies. Following on from the earlier observations of Rosen et al. (1980) that 1,25 $(OH)_2$ vitamin D levels are reduced in Pb-poisoned children, Markowitz et al. (1988) found that osteocalcin levels were inversely related to Pb body burden in moderately Pb-poisoned children. During chelation treatment for Pb, the osteocalcin levels were shown to increase.

An inverse relationship between blood Pb and stature and chest circumference was observed in children from the NHANES II study (Schwartz et al., 1986). There are several explanations for the inverse correlation between blood Pb and growth in children. First, blood Pb level may be a composite factor reflecting other genetic, ethnic, nutritional, environmental, and sociocultural factors. Second, nutritional deficits that retard growth also enhance Pb absorption. Finally, there may be a direct effect of low level Pb on growth in children. This

condition was explained by Dowd et al. (1994) as resulting from the inhibition by Pb^{2+} of binding of osteocalcin to hydroxyapatite. Effects similar to those described by Schwartz et al. (1986) were reported by Angle and Kuntzelman (1989), Lauwers et al. (1986), and Shukla et al. (1989).

Puzas et al. (1992) suggested Pb could upset the very sensitive interactive metabolic activity of osteoblasts and chondrocytes and thereby affect bone growth. In a later review, Puzas (2000) enlarged upon his earlier paper and described in more detail potential mechanisms of Pb effects on growth plate cartilage metabolism and on osteoclasts and osteoblasts, especially associated with osteoporosis.

Observational studies by Spencer et al. (1992, 1994) suggested a link between occupational Pb exposure and Paget's disease in both males and females, but the authors declined to advocate a causal effect. Later Spencer et al. (1995) found that 92% of a group of 48 patients with Paget's disease were exposed to Pb either from occupational or environmental sources. Adachi et al. (1998) explored a possible association between Pb and bone disease from XRF analyses of cortical and trabecular bone Pb content in 117 patients who attended a metabolic bone disease clinic (n = 92) or were undergoing dialysis for renal failure (n = 25). In patients suffering from Paget's disease, cortical bone Pb content was higher than it was in controls, patients with osteoporosis, and patients on dialysis. Trabecular bone Pb content was lowest in patients with Paget's disease or osteitis fibrosa. However, the authors could not distinguish between two alternatives, the first being that increased bone turnover due to Paget's disease releases Pb from trabecular bone that is then available for deposition into cortical bone, or secondly, that increased Pb content in cortical bone may cause increased turnover with release of Pb from trabecular bone.

In another facet of the Normative Aging Study, Shadick et al. (2000) investigated possible associations between long-term Pb accumulation and hyperuricemia and gouty arthritis in 777 male subjects. They found a positive association between patella bone Pb and uric acid levels ($p = 0.022$) but no association between bone or blood Pb and gout in this environmentally-exposed group.

6.9.6.3 Dental Health

Caries is considered an infectious disease arising from a multifactorial process involving particular flora, dietary exposures, and a susceptible host (Schafer and Adair, 2000). Increased caries risk has been detected in association with increasing blood Pb levels in populations with mean blood Pb concentrations of ~2–3 μg/dL (Dye et al., 2002; Gemmel et al., 2002; Moss et al., 1999).

Several studies have examined relationships between Pb exposure and the occurrence of dental caries in children and adults. The two largest studies were analyses of data collected in the NHANES III; both found significant associations between increasing caries prevalence and increasing blood Pb in children/adolescent (Moss et al., 1999) and adult (Dye et al., 2002) populations with geometric mean blood Pb levels of ~2.5 μg/dL. In the Moss et al. (1999) study, the odds ratios for caries in association with a 5 μg/dL increase in blood Pb concentration (i.e., from <2 μg/dL) was 1.8 (95% CI: 1.3, 2.5). Outcomes of two smaller studies were mixed, with one study finding no significant association between blood Pb and caries prevalence (Campbell et al., 2000) and the other finding significant associations (Gemmel et al., 2002); the latter being for children having a mean blood Pb concentration of 2.9 μg/dL (maximum 13).

The Moss et al. (1999) NHANES III analysis included results from coronal caries examinations on 24,901 subjects, stratified by age: 2 to 5 years (n = 3,547), 6 to 11 years (n = 2,894), and ≥12 years (n = 18,460). Specific outcomes assessed varied by age group: for children 2 to 11 years old who had at least one deciduous tooth, the number of deciduous teeth displaying decayed or filled surfaces (DFS); for subjects ≥6 years and who had at least one permanent tooth, the number of permanent teeth displaying decayed or filled surfaces; and for subjects ≥12 years, the sum of decayed, missing, and filled surfaces on permanent teeth (DMFS). In a multivariate linear regression model, increasing blood Pb concentration (log-transformed) was significantly associated with covariate-adjusted increases in dfs in the 2 to 5 year age group (β = 1.78 [SE 0.59], p = 0.004) and in the 6–11 year age group (β = 1.42 [SE 0.51], p = 0.007). Log-transformed blood Pb also was associated with increases in DFS in the 6–11 years age group (β = 0.48 [SE 0.22], p = 0.03) and in the ≥12 years age group (β = 2.50 [SE 0.69], p < 0.001), and increases in DMFS in the ≥12 years age group (β = 5.48 [SE 1.44], p = 0.01). The odds ratios (compared to 1st tertile, ≤1.66 μg/dL) for the binomial outcome, 0 or ≥1 DMFS, were 1.36 (95% CI: 1.01, 2.83) for the blood Pb range 1.66–3.52 μg/dL, and 1.66 (95% CI:

1.12, 2.48) for the range >3.52 μg/dL. Corresponding population risks attributable to blood Pb concentration were 9.6% and 13.5% in the blood Pb strata, respectively. An increase in blood Pb of 5 μg/dL was associated with an odds ratio of 1.8 (95% CI: 1.3, 2.5). Covariates included in the models were age, gender, race/ethnicity, poverty income ratio, exposure to cigarette smoke, geographic region, educational level of head of household, carbohydrate and calcium intakes, and frequency of dental visits.

Gemmel et al. (2002) conducted a cross-sectional study of associations between blood Pb concentration and dental caries in children, 6-10 years of age (n = 543), who resided either in an urban (n = 290) or rural (n = 253) setting. Mean blood Pb concentrations were 2.9 μg/dL (SD 2.0, maximum 13 μg/dL) in the urban group and 1.7 μg/dL (SD 1.0, maximum 7 μg/dL) in the rural group. Increasing blood Pb concentration (ln-transformed) was significantly associated with covariate-adjusted number of caries (dfs + DFS) (ln-transformed) in the urban group ($\beta = 0.22$ [SE 0.08], $p = 0.005$), but not in the rural group ($\beta = -0.15$ [SE 0.09], $p = 0.09$). When dfs counts were stratified by permanent or deciduous teeth, the blood Pb association in the urban group was significant for deciduous teeth ($\beta = 0.28$ [SE 0.09], $p = 0.002$), but not for permanent teeth ($\beta = 0.02$ [SE 0.07], $p = 0.8$). Covariates retained in the linear regression model were age, sex, ethnicity, family income, education of female guardian, maternal smoking, frequency of tooth brushing, firmness of toothbrush bristles, and frequency of chewing gum.

Campbell et al. (2000) was a retrospective cohort study in which dfs were assessed in children 7 to 12 years of age (n = 248) from Rochester, NY. Mean blood Pb concentration, measured at ages 18 and 37 months of age, was 10.7 μg/dL (range 18.0–36.8). The covariate-adjusted odds ratios for caries associated with a blood Pb concentration >10 μg/dL compared to ≤10 μg/dL were 0.95 μg/dL (95% CI: 0.43, 2.09) for permanent teeth and 1.77 μg/dL (95% CI: 0.97, 3.24) for deciduous teeth. Covariates retained in the logistic model were age, grade in school, number of tooth surfaces at risk. Other covariates examined in the models, all of which had no significant effect on the outcome, were gender, race/ethnicity, SES, parental education, residence in community supplied with fluoridated drinking water, and various dental hygiene variables. This study did not demonstrate that Pb exposure >10 μg/dL as a toddler was a strong predictor of caries among school-age children, but the authors noted that this might be due to limited statistical power.

Dye et al. (2002) analyzed data collected in NHANES III on indices of periodontal bone loss. The analysis was confined to subjects 20 to 69 years of age (n = 10,033). The geometric mean blood Pb concentration of the study group was 2.5 μg/dL (SE 0.08), with 2.4% of the group having blood Pb levels >10 μg/dL. Increasing log-transformed blood Pb was significantly associated with increasing prevalence of covariate-adjusted dental furcation ($\beta = 0.13$ [SE 0.05], $p = 0.005$). Dental furcation is indicative of severe periodontal disease. Covariates retained in the linear regression model were age, sex, race/ethnicity, education, smoking, and age of home. Smoking status was a significant interaction term when included in the model ($\beta = 0.10$ [SE 0.05], $p = 0.034$). When stratified by smoking status, the association between dental furcation and blood Pb concentration was significant for current smokers ($\beta = 0.21$ [SE 0.07], $p = 0.004$) and former smokers ($\beta = 0.17$ [SE 0.07], $p = 0.015$), but not for nonsmokers ($\beta = -0.02$ [SE 0.07], $p = 0.747$).

Some studies examined the relationship between tooth Pb levels and dental caries. In their compilation of metal concentrations in 1,200 deciduous teeth from a Norwegian population, Tvinnereim et al. (2000) found that carious teeth had higher Pb concentrations than noncarious teeth. Gil et al. (1994) measured Pb concentrations from 220 whole deciduous and permanent teeth from Coruna, Spain. The geometric mean Pb level was 10.36 μg/g of tooth. There was a significant increase in teeth Pb levels with advancing age. Permanent teeth showed higher mean Pb values (13.09 μg/g [SEM 1.07]) than deciduous teeth (3.96 μg/g [SEM 1.07]). The authors reported a possible relationship between increased Pb content and periodontal pathology but did not observe any relationship between Pb concentrations and caries.

6.9.7 Effects of Lead on Ocular Health

6.9.7.1 Summary of Key Findings of the Effects of Lead on Ocular Health from the 1986 Lead AQCD

The 1986 Lead AQCD did not address Pb effects on ocular health in humans. Various disturbances of the visual system have been observed in association with overt clinical Pb poisoning, including retinal stippling and edema, cataracts, ocular muscle paralysis, and impaired vision (see Otto and Fox, 1993 for review). Two longitudinal studies completed since 1986 provide evidence for (a) possible associations between Pb exposure and visual evoked retinal responses in children of mothers whose blood Pb levels in mid-pregnancy were in the 10 to

32 µg/dL range (Rothenberg et al., 2002b); and (b) a possible association between Pb exposure and risk of cataracts in males whose tibia bone Pb levels were in the 31 to 126 µg/g range (Schaumberg et al., 2004). These studies are summarized in Annex Table AX6-9.10.

6.9.7.2 Ocular Effects

In the Mexico City prospective Pb study, Rothenberg et al. (2002b) measured flash-evoked electroretinograms (ERG) in a subset of the study group (n = 45) at ages 7-10 years. As part of the prospective study, blood Pb concentrations had been measured during pregnancy and in the children, at birth and every 6 months, thereafter. Increasing maternal blood Pb, measured at 12 weeks of gestation, was significantly associated with increasing ERG a-wave and b-wave amplitude, with significant increases in a-wave in the second maternal blood Pb tertile (range 6.0–10.0 µg/dL), and a-wave and b-wave in the third maternal blood Pb tertile (range 10.5–32.5 µg/dL), compared to the first blood Pb tertile (range 2.0–5.5 µg/dL). No other blood Pb measurements were significantly associated with any ERG outcomes.

As part of the longitudinal Normative Aging Study, Schaumberg et al. (2004) analyzed prevalence of cataracts in adult males (n = 642), mean age 69 years (range 60–93 years). Subjects were stratified by blood Pb, patella bone Pb, or tibia bone Pb quintiles for a logistic regression analysis of the odds ratios for cataracts (first quintile as reference). Covariate adjusted odds ratio for cataracts in the fifth tibia bone Pb quintile was significant (3.19 [95% CI: 1.48, .90]). Odds ratios for cataracts were not significantly associated with patella bone Pb (1.88 [95% CI: 0.88, 4.02]) or blood Pb (0.89 [95% CI: 0.46, 1.72]). The first and fifth quintile Pb levels were 0–11 µg/g and 31–126 µg/g for tibia bone; 1–16 µg/g and 43–165 µg/g for patella bone; and 1.0–3.0 µg/g and 8–35 µg/dL for blood. Covariates retained in the regression model were age, smoking, history of diabetes; and daily intake of vitamin C, vitamin E, and carotenoids.

Cavalleri et al. (1982) measured visual fields of male workers exposed to Pb stearate in a polyvinyl pipe manufacturing facility (n = 35). Workers in a reference group (n = 350) were individually matched for age, smoking, and alcohol consumption. Visual sensitivity was significantly lower in Pb workers compared to the reference group; however, visual sensitivity index was not significantly associated with blood or urine Pb. Prevalence of mesopic scotoma (retinal light insensitivity under low illumination conditions) was 28.5% in the Pb workers and

0% in the reference group. Mean blood Pb levels were 46 μg/dL (range 21–82) in the Pb workers and 30 μg/dL (range 21–42) in the reference group.

6.9.8 Summary of the Epidemiologic Evidence for the Effects of Lead on Other Organ Systems

The following are a listing of key findings discussed above for Pb effects on other organ systems.

- ***Biochemical Effects of Lead.*** Evidence for disruption of heme synthesis derives from numerous studies in which Pb exposure has been associated with decreased activities of enzymes in the heme synthesis pathway (i.e., ALAS, ferrochelatase, cytochrome P450) and increased levels of substrates for heme synthesis (i.e., ALA, coproporphyrin, erythrocyte protoporphyrin) in both children and adults. Quantitative relationships between blood Pb concentration and the above biomarkers of impaired heme synthesis are highly consistent across studies. Increases in blood Pb concentration of ~20–30 μg/dL are sufficient to halve erythrocyte ALAD activity and sufficiently inhibit ferrochelatase to double erythrocyte protoporphyrin levels.

- ***Blood Lipids.*** Associations between occupational exposure to Pb and changes in blood lipid composition have been observed. These include increased levels of lipid peroxides in blood and/or serum, and increased serum levels of total and HDL cholesterol. Effects on serum cholesterol levels were evident in association with a blood Pb concentration in the range of 5–62 μg/dL (~mean 14 μg/dL). Oxidative changes in blood lipids (e.g., increased levels of lipid peroxides and malondialdehyde levels) as well as decreased levels of erythrocyte superoxide dismutase, catalase, G6PD, and GSH peroxidase; and increased lymphocyte reactive oxygen species and depleted GSH levels, indicative of increased oxidative stress, have been observed in Pb workers to be associated with blood Pb concentrations >30 μg/dL.

- ***Disruption of Hemoglobin Synthesis and Declines in Erythrocyte Numbers.*** Exposures that result in blood Pb concentrations <40 μg/dL appear to be tolerated without a decline in blood hemoglobin levels or hematocrit. However, perturbation of erythropoiesis, indicated by changes in serum erythropoietin and progenitor cells, occurs in association with blood Pb concentrations <40 μg/dL and in the absence of detectable changes in blood hemoglobin levels or hematocrit in children and adults. Risk of clinical anemia in children becomes appreciable at much higher blood Pb levels; a 10% decrease in hematocrit has been estimated to occur in association with blood Pb concentrations ≥85 μg/dL; a 10% probability of anemia (hematocrit <35%) was estimated to be associated with a blood Pb concentration of ~20 μg/dL at age 1 year, 50 μg/dL at age 3 years, and 75 μg/dL at age 5 years. In adults, with blood Pb levels below 25 μg/dL, increasing patella bone Pb, but not blood Pb, was associated with a significant decrease in hematocrit.

- ***Effects on the Endocrine System.*** Several studies have examined possible associations between Pb exposures in children and adults and various biomarkers of endocrine function, including the thyroid, male reproductive, and calcitropic endocrine systems. The strongest study designs have yielded no associations, or weak associations, between Pb exposure and thyroid hormone status. Studies of occupational exposures which included subjects having blood Pb concentrations >100 µg/dL have found depression of serum T3 and/or T4 levels, without a detectable increase in serum TSH; however, studies in which the blood Pb distribution was dominated by levels well below 100 µg/dL, have found either no effects or subclinical increases in serum T3, T4, with no change in TSH levels.

- ***Reproductive Endocrine Function.*** Studies of the male reproductive system that attempted to control for confounding effects of age have yielded mixed outcomes. Blood Pb ranges in these studies were similar (4–90 µg/dL), yet outcomes were mixed, with no change, or subclinical decrease in serum testosterone (TES) in association with Pb exposure. There are also mixed effects on serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) with increases, decreases, and no change in hormone levels observed. The inconsistency in the direction of effects on TES and the two androgen-regulating pituitary hormones, FSH and LH, is particularly noteworthy, in the absence of evidence for effects of Pb exposure on GnRH-induced FSH (Erfurth et al., 2001).

- ***Calcitropic Endocrine Function.*** Children exposed to relatively a high level of Pb (>30 µg/dL) may exhibit depressed levels of circulating 1,25-$(OH)_2D_3$. However, associations between serum vitamin D status and blood Pb may not be present in calcium-replete children who have average lifetime blood Pb concentrations below 25 µg/dL. In adults, exposures to Pb that result in blood Pb concentrations >40–60 µg/dL may increase, rather than decrease, circulating levels of 1,25-$(OH)_2D_3$ and PTH.

- ***Effects on the Hepatic System.*** Few studies of hepatic effects of Pb on humans have been reported since the 1986 Lead AQCD. Studies of hepatic enzyme levels in serum suggest that liver injury may be present in Pb workers; however, associations specifically with Pb exposures are not evident. Studies of urinary metabolites of cytochrome P450 phenotypes CYP2A6 and CYP3A4 suggest possible associations between Pb exposure and suppression of hepatic enzyme activity. The effect on CYP2A6 activity was observed in children with high Pb burdens (i.e., blood Pb >40 µg/dL, EDTA-provoked urinary Pb >500 µg/dL). The effect on CYP3A4 was observed in association with blood Pb ranges of ~30-112 µg/dL (based on reported serum Pb concentrations).

- ***Effects on the Gastrointestinal System.*** Several studies of prevalence of symptoms of GI colic in Pb workers provide evidence for symptoms in association with blood Pb levels >50–80 µg/dL. Similar types of studies of children have not been reported.

- ***Effect on Bone and Teeth.*** There is limited, but suggestive evidence of an association between Pb exposure and bone toxicity. However, in most studies, it is difficult to assess the direct contribution of Pb on bone diseases or reduced growth. Several studies that have explored possible associations between Pb exposure and risk of dental caries.

Increased caries risk has been detected in association with increasing blood Pb concentrations in populations with mean blood Pb concentrations of ~2-3 µg/dL.

- ***Ocular Health.*** Various disturbances of the visual system have been observed in association with overt clinical Pb poisoning, including retinal stippling and edema, cataracts, ocular muscle paralysis, and impaired vision. Two longitudinal studies completed since the 1986 Lead AQCD provide evidence for possible associations (a) between Pb exposure and visual evoked retinal responses in children of mothers with blood Pb concentrations in mid-pregnancy of 10.5–32.5 µg/dL and (b) between Pb exposure and risk of cataracts in middle-aged males whose tibia bone Pb levels were 31-126 µg/g.

6.10 EPIDEMIOLOGIC CONSIDERATIONS AND SUMMARY OF EVIDENCE FOR LEAD HEALTH EFFECTS

6.10.1 Introduction

A remarkable expansion has occurred since the 1990 Lead Supplement in the extent of the database available for drawing inferences about the various expressions of Pb toxicity. Moreover, the nature of the evidence available has changed as well. Many of the studies conducted prior to 1990 focused on the issue of whether an observed observation was likely to be real or the result of chance, selection bias, residual confounding, or some other methodological error. The validity of any association still needs to be ensured. The studies since 1990 mainly focus on characteristics of the pertinent concentration-response relationships (including the functional forms of the relationships), the slopes of the relationships, the natural histories of adverse effects, and the confounding or effect modifying influences of various co-exposures and host characteristics. Discussed below are pertinent issues that need to be considered in the evaluation and interpretation of the epidemiologic evidence regarding Pb health effects. Measurement error in the exposure and outcome variables are first discussed, followed by sections on potential confounding of Pb health effects and inferences of causality. Additional issues, including the concentration-response relationship and the persistence of Pb health effects are discussed in Chapter 8.

6.10.2 Exposure and Outcome Assessment in Lead Epidemiologic Studies

6.10.2.1 Assessment of Lead Exposure and Body Burdens Using Biomarkers

Blood and bone Pb levels serve as valuable indicators of Pb exposure in epidemiologic studies. Having a biomarker as a measure of pollutant exposure provides an important advantage beyond air lead measurements in estimating health effects. The expanded discussion below of the limitations in using biomarkers as indicators of exposure allows further understanding of the uncertainties potentially involved.

For any health endpoint of interest, the most useful biomarker of exposure is one that provides information about the Pb dose at the critical target organ and, moreover, reflects the exposure averaging time that is appropriate to the underlying pathogenetic processes (e.g., cumulative over lifetime, cumulative over a circumscribed age range, concurrent). In recent studies of Pb and health, the exposure biomarkers most frequently used are Pb in blood and bone (see discussion in Chapter 4). For outcomes other than those relating to hematopoiesis and bone health, these biomarkers provide information about Pb dose that is some distance from the target organ. For example, given that the central nervous system is considered the critical target organ for childhood Pb toxicity, it would be most helpful to be able to measure, in vivo, the Pb concentrations at the cellular site(s) of action in the brain. However, because such measurements are not currently feasible, investigators must rely on measurements of Pb in the more readily accessible but peripheral tissues. The relationship between brain Pb and Pb in each of these surrogate tissues is still poorly understood, although the pharmacokinetics clearly differs among these compartments. In both rodents and nonhuman primates, brain Pb level falls much more slowly than blood Pb level following chelation with succimer and, in the rodent, in nonchelated animals after cessation of exposure. These observations suggest that using blood Pb as an index of Pb in the brain will result in exposure misclassification, although the magnitude of this bias in any specific setting will be difficult to characterize. The most likely direction, however, would be underestimation of the amount of Pb in the brain, at least under scenarios involving chronic exposure.

As an exposure biomarker, blood Pb level has other limitations. Only about 5% of an individual's total body Pb burden resides in blood. Furthermore, blood consists of several subcompartments. More than 90% of Pb in whole blood is bound to red cell proteins such as hemoglobin, with the balance in plasma. From a toxicological perspective, the unbound fraction

is likely to be the most important subcompartment of blood Pb because of the ease with which it diffuses into soft tissues. The concentration of Pb in plasma is much lower than in whole blood, however. For example, in a group of pregnant women with blood Pb levels below 10 μg/dL, plasma Pb levels were less than 0.3% of the whole blood Pb level. The greater relative abundance of Pb in whole blood makes its measurement much easier (and more affordable) than the measurement of Pb in plasma. The use of whole blood Pb as a surrogate for plasma Pb could be justified if the ratio of whole blood Pb to plasma Pb were well characterized, but this is not so. At least some studies suggest that it varies several-fold among individuals with the same blood Pb level. Moreover, the ability of red cells to bind Pb is limited, so the ratio of blood Pb to plasma Pb would be expected to be nonlinear. Thus, interpreting whole blood Pb level as a proxy for plasma Pb level, which, itself, is a proxy for brain Pb level, will result in some exposure misclassification.

Although the use of blood Pb may not best reflect the actual dose of Pb in the specific target organs of interest, of greater concern are the epidemiologic implications of its use. In a regression model, the variation in Pb about its mean is correlated with the variation in outcome about its mean. Because only variations about the mean contribute to the association, mean differences between true and estimated levels become irrelevant. The measurement error in considering blood Pb as a surrogate for brain Pb will bias the blood Pb effect towards the null and is an example of classical measurement error. The error will be nonlinear if red cell binding is limited. However, when interest centers on the low blood Pb level, the error should be approximately additive, multiplicative, or both. Another example of measurement error with epidemiologic implications is the Berksonian error. Berkson error arises when averages of blood Pb levels are used in a regression rather than individual data. For example, if a regression of IQ is performed on averages of children's blood Pb concentrations grouped between intervals, the Berkson error model will apply. The slope will be unbiased, but the standard errors will be inflated.

There are additional issues to consider in the use of blood as a marker of Pb exposure. The residence time of Pb in blood is closely linked to red cell lifetime, with a half-time on the order of 30 days. Thus, a high blood Pb level does not necessarily indicate a high body Pb burden. Similarly, individuals who have the same blood Pb level will not necessarily have similar body burdens or exposure histories. The rate at which blood Pb level changes with

time/age depends on exposure history due to re-equilibration of Pb stored in the various body pools. In nonchelated children, the time for blood Pb to decline to a value less than 10 μg/dL was linearly related to baseline blood Pb level. A single blood Pb measurement might therefore provide limited information about an individual's Pb exposure history, a difficulty frequently cited with respect to the interpretation of cross-sectional studies of pediatric Pb toxicity, in which children's blood Pb level is often measured only once, and sometimes only well after the period when blood Pb levels typically peak (18–30 months of age). If it is exposures to Pb in the early postnatal years that are most detrimental to children's development, categorizing a child's exposure status based on a blood Pb level contemporaneous with the measurement of neurodevelopment at school-age could result in exposure misclassification. This concern must be qualified, however, by recent data from some longitudinal studies indicating that concurrent blood Pb level, even at ages well beyond 18 to 30 months, is sometimes the strongest predictor of late outcomes (Canfield et al., 2003a; Dietrich et al., 1993a,b; Tong et al., 1996; Wasserman et al., 2000b). Changes in blood Pb concentration in children are found to closely parallel changes in total Pb body burden. Empirical evidence in support of this comes from longitudinal studies in which relatively high correlations ($r = 0.85$) were found between concurrent or lifetime average blood Pb concentrations and tibia bone Pb concentrations (measured by XRF) in a sample of children in which average blood Pb concentrations exceeded 20 μg/dL; the correlations were much weaker ($r = <0.15$) among children who had average blood Pb concentration ≤ 10 μg/dL (Wasserman et al., 1994).

Age-related changes in vulnerability, and the reasons why it might differ across studies, remain uncertain. It may be that among children with chronically elevated exposure, but not in those with relatively low lifetime exposure, blood Pb level measured at school-age is a reasonably good marker of cumulative exposure. That concurrent blood Pb level is, under some circumstances, a stronger predictor of school-age outcomes than is blood Pb level in the early postnatal years does not necessarily imply greater vulnerability of the brain to ongoing than to past exposure. Due to the high intercorrelation among blood Pb measures taken at different time points, it is not feasible to examine exposures during any given age for evidence of a sensitive neurodevelopmental period.

The development of X-ray-fluorescence (XRF) methods for measuring Pb in mineralized tissues offers another approach for characterization and reconstruction of exposure history. Such

tissues are long-term Pb storage sites, with a half-life measured in decades and contain ~90% of the total body Pb burden in adults and 70% in children. Thus, bone Pb is an index with a long exposure averaging time. XRF methods have proven useful in studying individuals with occupational Pb exposure, those living in highly polluted environments, and those for whom community Pb exposures are or, in the past, were relatively high (e.g., Korrick et al., 1999; Schwartz et al., 2000a,b,c,d). In a relatively highly exposed cohort of pregnant women in Mexico City, higher bone Pb levels at one month postpartum were associated with reduced birth weight, less infant weight gain, smaller head circumference and birth length, and slower infant development (Gomaa et al., 2002; González-Cossio et al., 1997; Hernandez-Avila et al., 2002; Sanín et al., 2001). Among children living near a large Pb smelter in Yugoslavia, IQ at age 10-12 years was more strongly associated, inversely, with tibia Pb level than with blood Pb level (Wasserman et al., 2003).

Current XRF methods for measuring bone Pb levels have limitations, however. Temporal features of exposure history cannot readily be discerned. Some progress has been made toward this goal by examining the spatial distribution of Pb in teeth in relation to the relative abundance of stable Pb isotopes, but the specialized technologies needed to carry out these analyses are unlikely ever to be widely available, and the unpredictability of tooth exfoliation makes this tissue difficult to collect unless the study design involves contact with (and the cooperation of) participants at the appropriate ages. Current XRF methods might not be sufficiently sensitive for studies of the health effects of low-dose community exposures. The bone Pb levels of a large percentage of subjects might be below the detection limit, e.g., 80% in a case-control study of bone Pb levels and juvenile delinquency in which the minimum detection limit was 21.5 μg/g bone mineral (Needleman et al., 2002). Even among individuals known to have histories of substantial Pb exposures, such as adolescents and young adults who grew up near the Bunker Hill smelter in Idaho (McNeill et al., 2000), bone Pb levels tend to be low. Lead appears to be deposited at sites of most active calcification. In children, this is trabecular bone, in which the rate of fractional resorption in early childhood is high. Depending on the amount of the child's ongoing exposure, Pb deposited in bone might not remain there for decades, making bone Pb level an imprecise index of lifetime Pb exposure. This concern also exists in the use of tooth Pb to represent cumulative Pb exposure in children. Rabinowitz et al. (1993) observed that a child's tooth Pb level was more strongly related to blood Pb level around the time of tooth exfoliation

than to an integrated index of blood Pb level prior to exfoliation. Finally, it is difficult to compare the performance of different laboratories using XRF methods to measure bone Pb because of the absence of standard reference materials. Nevertheless, efforts continue to modify the instrumentation or measurement protocols to reduce the detection limit.

A major research need is the development and validation of biomarkers of critical dose that, compared to blood Pb or bone Pb, are fewer toxicokinetic steps removed from the sites of Pb's actions in the brain. One promising front in the effort to deduce the contents of the "black box" separating external dose and clinical disease is the measurement of processes and products that potentially mediate the association between them. For example, magnetic resonance spectroscopy (MRS) has been used in small case series to measure the ratio of N-acetylaspartate to creatine, which are a marker of neuronal and axonal damage and thus, an early biological effect rather than a biomarker of exposure. In children, higher Pb exposures are associated with lower N-acetylaspartate to creatine ratios in the frontal gray matter and, to a lesser extent, in frontal white matter (Trope et al., 1998, 2001). Similarly, an adult who had higher bone and blood Pb levels than did his monozygotic twin had both greater neuropsychological deficits and lower N-acetylaspartate to creatine ratios in the hippocampus, frontal lobe, and midbrain (Weisskopf et al., 2004b). While much remains uncertain about the interpretation of MRS, the use of this and other biochemical imaging methods, in combination with more conventional structural and functional imaging methods, may improve the current understanding of the mechanisms of Pb neurotoxicity.

Despite these limitations, blood and bone Pb levels both provide relevant and valuable measures of Pb exposure in epidemiologic studies. Strong and consistent associations have been observed in the epidemiologic literature between blood and/or bone Pb levels and various health effects, most notably for neurotoxicity in children and cardiovascular effects in adults. Evaluation of relative strength of associations of particular health endpoints with blood versus bone Pb measures has been useful in a number of studies in attributing the relative likelihood of effects being due to concurrent recent Pb exposures versus past peak or long-term cummulative Pb exposures. Given the number of toxicokinetic steps separating Pb levels at the critical target organs from the usual exposure biomarkers, the progress made in characterizing concentration-response relationships for various Pb-related health outcomes is remarkable.

6.10.2.2 Assessment of Health Outcomes

Outcome measurement and outcome classification have generally received less attention from investigators than have exposure measurement and misclassification. The specific problems are, to some extent, endpoint domain-specific. With regard to neurodevelopmental toxicities, critical issues are whether the assessment instruments used are psychometrically sound and appropriate for the study cohort, the data generated will support adequate tests of the study hypotheses, and whether the instruments have been administered and scored consistently and correctly. With regard to the cardiovascular toxicities of increased blood pressure/prevalence of hypertension, the critical issue is whether the blood pressure value recorded for a participant is an accurate estimate. Multiple measurements of blood pressure are frequently made in a study but investigators usually have not taken advantage of the collected information to quantify the amount of error in the measurements. This information can be used to improve the reliability of the measurements, which would be expected to improve the precision of the associations estimated. Similarly, aggregating scores to estimate latent variables representing, for instance, "language skills" or "visual-spatial skills" is an approach that might take advantage of the overlapping information provided by the multiple tests included in neurobehavioral test batteries, producing more reliable endpoint variables. This approach, however, has not been widely applied in Pb studies. Concerns regarding the presence of measurement error in the outcome variable need to be considered in the context of the exposure of interest. If the measurement error in outcome is uncorrelated with exposure, it will not induce bias in the estimate of the effect of Pb. However, it should be noted that the measurement error will lead to reduced power to detect a significant effect.

6.10.3 Confounding of Lead Health Effects

6.10.3.1 Methods Used to Adjust for Confounding in Epidemiologic Studies of Lead

The possibility that the adverse health effects associated with increased Pb exposure in epidemiologic studies are, in fact, due to risk factors with which increased Pb exposure is associated remains the most important impediment to drawing causal inferences. It is important to note that confounding is not an inherent characteristic of an association between Pb exposure and a health outcome. Rather it is a bias that arises from the particular setting in which the association is being investigated, and its source is the patterns of covariance between Pb, the

outcome, and other determinants of the outcome. Therefore, the extent to which it represents an interpretational challenge is, to some extent, study-specific. Various approaches have been taken to reduce the uncertainty this creates. Some investigators have specified the sampling frame or the eligibility criteria so as to increase the homogeneity of the study participants on factors known to be strong risk factors for the outcome of interest, thereby reducing both (a) the correlation between them and Pb and (b) their potential to confound any association observed between increased Pb exposure and poor outcome. An example is the recruitment of a birth cohort from a maternity hospital that largely served a relatively affluent catchment area, resulting in high umbilical cord blood Pb levels being associated with higher, rather than lower, social class standing (Bellinger et al., 1984). Reducing confounding by means of such design decisions has the disadvantage that an investigator cannot determine whether the impact of Pb on the outcome varies depending on the factor whose range of potential values has been restricted. More frequently, however, investigators have relied on statistical procedures, applied post data collection, to identify and control for potential confounding. Unlike sample restriction, this approach preserves the opportunity to explore possible modification of the Pb effect by cofactors.

Adjustment for confounding has been performed primarily using multiple regression analyses and data stratification. For multiple regression modeling, stepwise regression has been frequently used for covariate selection. Stepwise regression has many faults and is often less acceptable then the use of a few well-chosen covariates. However, the stepwise regression methodology may be considered to have less bias, as it selects from a class of variables that represent a wide scientific viewpoint rather than the narrower one of the investigator. One problem with stepwise regression pointed out by Bellinger (2004) is that the usual adjustment strategy assumes that all the variance in the response shared by the exposure and the confounder belongs to the confounder. In some settings, this is likely to be excessively conservative, because confounders can, to some extent, also be proxies for exposure. This is further discussed in the next section.

Splitting the data set into smaller data sets (partitioning or stratification) and analyzing those data sets separately was used in some of the studies examining the relationship between blood pressure and Pb. This practice also has some advantages and disadvantages. Use of an advanced statistical method could be helpful to determine how the partitioning should be done

(Young and Hawkins, 1998), which could reveal relationships that would otherwise not be possible to detect using usual regression techniques. A disadvantage of partitioning a small data set is that the smaller sample size may lack sufficient power to detect otherwise detectable associations and to yield reliable estimates.

6.10.3.2 Effects of Confounding Adjustment on Lead Health Effect Estimates

The ability of the investigator to determine how much of the apparent association between a Pb biomarker and an outcome reflects residual confounding by a cofactor depends on the characteristics of the joint distribution of Pb and the cofactor. For example, with respect to neurodevelopment, important cofactors include maternal IQ, quality of the rearing environment, maternal smoking, alcohol use, and birth weight, among others. Some of these cofactors are truly independent predictors and can be adjusted for using multiple regression analyses. Under some circumstances, however, Pb and the cofactor may be so highly related that one cannot be confident that their associations with the outcome have been disentangled by the statistical methods applied. Moreover, the true causal relationships among Pb, the cofactors, and the outcome might not be sufficiently well understood that the outcome variance shared by Pb and the cofactors can be characterized appropriately in the analyses.

In studies of Pb and neurodevelopment, the magnitude of the Pb coefficient, reflecting the decline in test score per unit increase in the Pb biomarker, is substantially reduced, often by half or more, by adjusting for markers of the social environment. However, as noted above, the extent of confounding is study-specific, so the impact of adjustment for confounders on the Pb coefficient is also study-specific. With respect to the Port Pirie study, Tong and Lu (2001) observed that adjustment for four factors (i.e., quality of home environment, SES, maternal intelligence, and parental smoking behavior) reduced the magnitude of the estimated association between Pb and IQ by 40% and inclusion of additional factors resulted in another 10% reduction. Similarly, in the pooled analysis by Lanphear et al. (2005) that included seven prospective studies, the crude coefficient for concurrent Pb and childhood IQ score was −4.66 (95% CI: −5.72, −3.60), while the coefficient adjusted for study site, quality of the home environment (HOME score), birth weight, maternal IQ, and maternal education was −2.70 (95% CI: −3.74, −1.66). When expressed as the percentage of variance accounted for in a health outcome, the contributions of Pb have been characterized as modest in magnitude. For example, Koller et al.

(2004) noted that blood Pb typically accounts for 1 to 4% of the variance in child IQ scores, compared to 40% or more by social and parenting factors. During the 1980s, adjustment for parental IQ and HOME scores became almost mandatory if the findings of a study of Pb and children's cognitive outcomes were to be considered credible. Simulation analyses conducted by Mink et al. (2004) suggested that relatively small differences in confounding variables between "exposed" and "unexposed" groups could produce spurious differences in cognitive test scores if unmeasured and unaccounted for in the analysis. As noted by Bellinger (2004), however, the problem usually is not that such cofactors were unmeasured in a Pb study, but that they were not measured well.

More important yet is the fact that the conceptual models that frame the interpretation of the resulting models usually fail to reflect adequately the complexity of the associations among Pb exposure, the outcome, and the cofactors. Although both HOME score and parental IQ surely strongly influence child outcomes in ways that are independent of Pb, a case can also be made that Pb might contribute to the associations. That is, a parent's IQ presumably reflects the parent's early Pb exposure and, assuming that the physical environments in which a parent and child grow up are not completely unrelated to one another are likely to provide similar Pb exposure opportunities. Adjusting for parental IQ in evaluating the association between a child's Pb exposure and his or her IQ, therefore, will result in an underestimate of the contribution of the child's Pb exposure to his or her IQ. Similarly, if early Pb exposure alters child behavior, the transactional model of child development would generate the prediction that the changes will elicit different behaviors from parents, altering the characteristics of the child-rearing environment. For instance, increased Pb exposure might result in an infant being more irritable, less soothable, and the parent less nurturing. In so far as measurement of the quality of the rearing environment in studies occurs after the children have experienced some Pb exposure, the hypothesis that Pb is responsible for shaping some aspects of that environment cannot be entirely dismissed, and control for HOME scores might be excessively conservative.

Other aspects of model building in assessing the association of Pb with health outcomes also warrant comment. In many studies of Pb and cognitive outcomes in children, investigators have adjusted for factors such as birth weight or length of gestation that might, themselves, reflect adverse effects of Pb, i.e., mediating factors that lie between Pb and condition on the causal pathway. The coefficient estimated for Pb in a model that contained such factors would

be smaller in magnitude than it would be if terms for such mediating factors had not been included.

Recognizing imperfections in the ability to measure such factors well, a concern is expressed that the Pb coefficient could be reduced further, perhaps all the way to the null, if better, more comprehensive methods of measurement were applied. On the other hand, the methods used to adjust for such factors may be excessively conservative insofar as they attribute to a factor all of the outcome variance that it shares with Pb, despite the likelihood that the true relationships among Pb, social factors, and outcome are unlikely to be as simple as this model assumes. Some factors might, in part, be markers of Pb exposure opportunities. For example, both Pb biomarker levels and lower cognitive function in children are associated with lower social class standing. Social class is a complex construct that conveys information about a multitude of factors that might influence children's health, including the amount of Pb in environmental media. Thus, some of the association between lower social class and poorer health might reflect the effect of higher Pb exposure. If so, routine adjustment of health outcome for social class in assessing the association between increased Pb exposure and poorer health in children will fail to distinguish these Pb-related and non-Pb-related components of the association between social class and health, and, in fact, will assume that all of it is non-Pb-associated (Bellinger et al., 1989). It is nearly impossible to actually determine if the problem of overadjustment exists in a particular data set. There are several statistical methods which attempt to address this problem. These include using partial F tests, ridge regression, path analysis, and structural equations. None of these methods are completely satisfactory.

6.10.4 Inferences of Causality

Even with more sophisticated and nuanced models, however, any conclusions about the causal forces generating the results of any observational epidemiologic study are necessarily uncertain. In the absence of random assignment to exposure group, residual confounding will always be a possible explanation of an observed association. As in other areas of epidemiology, a weight-of-evidence approach remains the best option available as a basis for drawing of causal inferences. If the association between a Pb biomarker and a health outcome of interest is observed in settings that vary widely in terms of the characteristics of the social environment including sociodemographic and cultural characteristics, characteristics of the study participants,

including nutritional status, genetic factors, and lifestyle factors, the likelihood that the association is attributable, in its entirety, to residual confounding is reduced. For instance, the pooled analyses of data contributed by many of the international prospective studies provide a compelling demonstration that the association between blood Pb level and child IQ is remarkably robust across disparate sociocultural settings (Lanphear et al., 2005). Even such consistency in the effect estimate across diverse settings is only indirect and weak evidence of causality, however. In general, epidemiologic studies rarely provide data that enhance understanding of the "black box" between biomarkers of Pb burden and indicators of health status. Epidemiologic data identify associations between exposure biomarkers and health indicators, but are not highly informative regarding possible mechanisms of Pb toxicity that underlie the associations.
A critical stage in applying the overall weight-of-evidence approach is the examination of the epidemiologic data in the context of data from experimental animal behavioral and mechanistic studies. Although such data have their own limitations, they are not subject to many of the most important potential biases that can becloud the interpretation of the epidemiologic data.

6.10.5 Summary of Key Findings and Conclusions Derived from Lead Epidemiology Studies

The remarkable progress made since the mid-1980s in understanding the effects of Pb on health can be gauged by noting the changes that have occurred over time in the questions that investigators have addressed. In the 1980s, the question of interest was often, "Does low-level Pb exposure affect health?" The questions asked in more recent studies have more often focused on details of the associations, including the shapes of concentration-response relationships, especially at levels well within the range of general population exposures, biological and socioenvironmental factors that either increase or decrease an individual's risk, the prognoses associated with Pb-associated effects, the efficacy of interventions to reduce adverse effects, and so on. In fact, "low-level," a term long-used to describe exposures not sufficiently high to produce clinical signs and symptoms, is increasingly being recognized as a descriptor that has little biological meaning and is interpretable only in a specific historical context. What was considered "low" in the 1980s is an order of magnitude higher than the current mean blood Pb level in the U.S. population, and the current mean remains perhaps as much as two orders of magnitude above "natural" background blood Pb levels in humans. The current CDC screening

guideline for children of 10 µg/dL is not a "bright line" separating toxicity from safety, but merely a risk management tool. There is no level of Pb exposure that can yet be clearly identified, with confidence, as clearly not being associated with potentially increased risk of deleterious health effects. Recent studies of Pb neurotoxicity in children consistently indicate that blood Pb levels <10 µg/dL are associated with neurocognitive deficits. The data are also suggestive that these effects may be seen at blood Pb levels ranging down to 5 µg/dL, or perhaps somewhat lower, but the evidence is less definitive. Public health interventions have resulted in declines over the past 25 years of more than 90% in the mean blood Pb level within all age and gender subgroups of the U.S. population, substantially decreasing the numbers of individuals at risk for toxic effects of Pb. The following provides a listing of most salient key findings for various classes of health outcomes discussed in this chapter:

- **Neurotoxic effects of lead in children.** Lead effects on neurobehavior in children have been observed with remarkable consistency across numerous studies of various designs, populations, and developmental assessment protocols. The negative impacts of Pb on neurocognitive ability and other neurobehavioral outcomes persist in most recent studies even after adjustment for numerous confounding factors, including social class, quality of caregiving, and parental intelligence. These effects appear to persist into adolescence and young adulthood. Collectively, the prospective cohort and cross-sectional studies offer evidence that exposure to Pb affects the intellectual attainment of preschool and school age children at blood Pb levels <10 µg/dL (most clearly in the 5 to 10 µg/dL range, but, less definitively, possibly lower). Epidemiologic studies have demonstrated that Pb may also be associated with increased risk for antisocial and delinquent behavior, which may be a consequence of attention problems and academic underachievement among children who may have suffered higher exposures to Pb during their formative years. Direct measures of brain damage using Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) also provide evidence suggestive of neurologic harm due to Pb exposure. Also, pharmacological or nutritional intervention strategies generally have not been found to be effective in reducing or eliminating Pb-associated neurodevelopmental morbidities in the absence markedly reduced environmental exposures.

- **Neurotoxic effects of lead in adults.** In the limited literature examining environmental Pb exposure, mixed evidence exists regarding associations between Pb and impaired cognitive performance in adults. Studies using concurrent blood Pb levels as the marker for Pb exposure found no association between cognitive performance and Pb exposure. However, significant associations were seen in relation to bone Pb concentrations, suggesting that long-term cumulative exposure may be crucial in contributing to neurocognitive deficits in adults. Numerous studies of occupational Pb exposure observed associations of blood Pb with peripheral sensory nerve impairment, visuomotor and memory impairment, and postural sway abnormalities. Past high-level occupational Pb exposures have also been associated with increased risk of developing amyotrophic lateral sclerosis (ALS), motor neuron disease, and essential tremor. The odds of developing ALS and essential tremor were significantly increased in individuals with the ALAD2 allele. These neurobehavioral impairments in occupationally-exposed individuals have typically been associated with notably elevated blood Pb levels (~30 to 40 µg/dL); however, essential tremor has been found to be associated with much lower blood Pb levels (mean 3 µg/dL).

- **Renal effects of lead.** In the general population, both cumulative and circulating Pb has been found to be associated with longitudinal decline in renal functions. In the large NHANES III study, alterations in urinary creatinine excretion rate (one indicator of possible renal dysfunction) was observed in hypertensives at a mean blood Pb of only 4.2 µg/dL. These results provide suggestive evidence that the kidney may well be a target organ for effects from Pb in adults at current U.S. environmental exposure levels. The magnitude of the effect of Pb on renal function ranged from 0.2 to -1.8 mL/min change in creatinine clearance per 1.0 µg/dL increase in blood Pb in general population studies. However, the full significance of this effect is unclear, given that other evidence of more marked signs of renal dysfunction have not been detected at blood Pb levels below 30-40 µg/dL among thousands of occupationally-exposed Pb workers that have been studied. The renal impact of environmental Pb exposure in children is difficult to assess, because most studies have only measured early biological effect markers and their prognostic value is uncertain. Studies involving the longitudinal assessment of renal

function decline in susceptible patient populations have observed that low levels of blood Pb (<5 µg/dL) and chelatable Pb levels were associated with decline in glomerular filtration rate over a 4-year follow-up period in patients with chronic renal insufficiency.

- **Cardiovascular effects of lead.** Epidemiologic studies support the relationship between increased Pb exposure and increased deleterious cardiovascular outcomes, including increased blood pressure and increased incidence of hypertension. A recent meta-analysis reported that a doubling of blood Pb level was associated with a 1.0 mm Hg increase in systolic blood pressure and a 0.6 mm Hg increase in diastolic pressure. Studies also have found that cumulative past Pb exposure (as indexed by bone Pb) may be as important, if not more, than present exposure in assessing cardiovascular effects. The evidence for an association of Pb with cardiovascular morbidity and mortality is limited but supportive.

- **Reproductive and developmental effects of lead.** The epidemiologic evidence suggests small associations between exposure to Pb and male reproductive outcomes, including perturbed semen quality and increased time to pregnancy. These associations appear at blood Pb levels >45 µg/dL, as most studies have only considered exposure in the occupational setting. There are no adequate data to evaluate associations between Pb exposure and female fertility. For many other outcomes, the observed associations are fairly small, especially at the levels of exposure that are currently of interest. However, there may be populations that are highly susceptible to Pb-related reproductive effects, especially if they have additional risk factors for these outcomes.

- **Genotoxic and carcinogenic effects of lead.** Studies of genotoxicity consistently find associations of Pb exposure with DNA damage and micronuclei formation; however, the associations with the more established indicator of cancer risk, chromosomal aberrations, are inconsistent. Epidemiologic studies of highly-exposed occupational populations suggest a relationship between Pb and cancers of the lung and the stomach; however the evidence is limited by the presence of various potential confounders, including coexposures (e.g., to arsenic and/or Cd), smoking, and dietary habits. The 2003 NTP and 2004 IARC reviews concluded that Pb and Pb compounds were probable carcinogens based on limited evidence in humans and sufficient evidence in animals. Similarly,

Pb compounds would likely be classified as probable human carcinogens according to U.S. EPA (2005) Cancer Guidelines based on experimentally demonstrated carcinogenic effects in animals, although the human evidence would be considered inadequate according to those new 2005 guidelines.

- **Effects of lead on the immune system.** Several studies have examined possible associations between Pb exposures and biomarkers of immune function. Findings from recent epidemiologic studies suggest that Pb exposure may be associated with effects on cellular and humoral immunity. These effects include changes in serum immunoglobulin levels; perturbation of peripheral lymphocyte phenotype profiles, including decreases in peripheral blood T-cell abundance and changes in T-cell to B-cell abundance ratios; suppression of lymphocyte activation; and suppression of neutrophil chemotaxis and phagocytosis. Studies of biomarkers of humoral immunity in children have consistently found significant associations between increasing blood Pb concentrations and serum IgE levels at blood Pb levels <10 μg/dL.

- **Effects of lead on the hematopoietic system.** Lead exposure has been associated with disruption of heme synthesis in both children and adults. Increases in blood Pb concentration to ~20 to 30 μg/dL are sufficient to halve erythrocyte ALAD activity and sufficiently inhibit ferrochelatase to double erythrocyte protoporphyrin levels. Perturbation of erythropoiesis, indicated by changes in serum erythropoietin and progenitor cells, occurs in the absence of detectable changes in blood hemoglobin levels or hematocrit in children and adults at blood Pb levels <40 μg/dL. A 10% probability of anemia (hematocrit <35%) is estimated to be associated with a blood Pb level of ~20 μg/dL at age 1 year.

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7. ENVIRONMENTAL EFFECTS OF LEAD

7.1 TERRESTRIAL ECOSYSTEMS

Surface soils across the United States are enriched in lead (Pb) relative to levels expected from natural (geogenic) inputs (Erel and Patterson, 1994; Francek, 1992; Friedland et al., 1984; Marsh and Siccama, 1997; Murray et al., 2004; Yanai et al., 2004). While some of this contaminant Pb is attributed to paint, salvage yards, shooting ranges, and the use of Pb arsenate as a pesticide in localized areas (Francek, 1997), Pb contamination of surface soils is essentially ubiquitous because of atmospheric pollution associated with iron and steel foundaries, boilers and process heaters, the combustion of fossil fuels in automobiles, trucks, airplanes, and ships, the manufacturing of cement, and other industrial processes (Table 2-8, Newhook et al., 2003; Polissar et al., 2001). However, Pb inputs to terrestrial ecosystems in the United States have declined dramatically in the past 30 years. The primary reason for this decline has been the almost complete elimination of alkyl-Pb additives in gasoline in North America. Also, emissions from smelters have declined as older plants have been shut down or fitted with improved emissions controls.

Most terrestrial ecosystems in North America remain sinks for Pb, despite reductions in atmospheric Pb deposition of more than 95%. Lead released from forest floor soils in the past has been largely immobilized in mineral soils (Miller and Friedland, 1994; Johnson et al., 1995, 2004; Kaste et al., 2003; Watmough et al., 2004). The amount of Pb that has leached into the mineral soil to date ranges from 20 to 90% of the total anthropogenic Pb deposition, depending on forest type, climate, and litter cycling. While inputs of Pb to ecosystems are currently low, Pb export from watersheds via groundwater and streams is substantially lower than inputs. Reported concentrations of Pb in waters draining natural terrestrial ecosystems have always been low (Bacon and Bain, 1995; Johnson et al., 1995b; Wang et al., 1995; Vinogradoff et al., 2005), generally less than 1 ng L^{-1}, even at moderately polluted sites (Laskowski et al., 1995). Thus, even at current input levels, watersheds are accumulating industrial Pb (Wang et al., 1995; Scudlark et al., 2005). However, burial/movement of Pb over time down into lower soil/sediment layers also tends to sequester it away from more biologically active parts of the watershed (unless later disturbed or redistributed, e.g., by flooding, dredging, etc.).

The current chapter first summarizes the most relevant information from the 1986 Lead Air Quality Criteria Document (Lead AQCD) (U.S. Environmental Protection Agency, 1986) and then assesses new information that has become available on the potential effects of atmospheric Pb inputs on the terrestrial ecosystem. It has been organized to address: methodologies used in terrestrial ecosystem research (Section 7.1.1); the distribution of atmospherically delivered Pb in terrestrial ecosystems (Section 7.1.2); Pb uptake and mechanisms of action (Section 7.1.3); toxic effects of Pb on terrestrial organisms (Section 7.1.4); and, Pb effects on natural terrestrial ecosystems (Section 7.1.5). The major findings and conclusions from each corresponding Annex section for these subject areas are summarized here.

7.1.1 Methodologies Used in Terrestrial Ecosystem Research

Several methodologies used in terrestrial ecosystems research are described in Annex Section AX7.1.1, with additional discussion in AX7.1.2 of the application of these methods to the study of the distribution of atmospherically delivered Pb. One of the key factors necessary for understanding ecological risks is related to bioavailability. The National Research Council (NRC) 2002 review on bioavailability defined the "bioavailability processes" in terms of three key processes. One of these processes, contaminant interactions between phases, is more commonly referred to as "speciation." For a given metal or metalloid, the term speciation describes the chemical's ability to interact with its biological or chemical surroundings by characterizing its physicochemical properties that are relevant to bioavailability.

Methods to address bioavailability (speciation), and methods used to reduce Pb bioavailability, are summarized in this section.

Analytical Tools and Models

A wide variety of analytical tools have been used to characterize a metal's speciation as it is found in various media:

- XRD - X-ray diffraction;
- EPMA - electron probe microanalysis;
- PIXE and μPIXE - particle induced X-ray emission;
- XPS - X-ray photoelectron spectroscopy;
- XAS - X-ray absorption spectroscopy;

- SIMS - secondary ion mass spectrometry;
- sequential extractions; and,
- single chemical extractions.

EPA techniques provide the greatest information on metal speciation. Other techniques, such as EXAFS (extended X-ray absorption fine structure) and EXANES (extended X-ray absorption near edge spectroscopy), show great promise and will be important in solving key mechanistic questions. In the case of phytotoxicity, the speciation of metals by direct measurement or chemical models of pore water chemistry is most valuable.

The tools that have been used most often to evaluate speciation for metal particles in various media include the following computer-based models: SOILCHEM, MINTEQL, REDEQL2, ECOSAT, MINTEQA2, HYDRAQL, PHREEQE, and WATEQ4F.

Metal Speciation for Plants

When considering the bioavailability of a metal to plants from soils and sediments, it is generally assumed that both the kinetic rate of supply and the speciation of the metal to either the root or shoot are highly important. In soils and sediments, generally only a small volume of water is in contact with the chemical form, and although the proportion of a metal's concentration in this pore water to the bulk soil/sediment concentration is small, it is this phase that is directly available to plants. Therefore, pore water chemistry (i.e., metal concentration as simple inorganic species, organic complexes, or colloid complexes) is most important.

Tools currently used for metal speciation for plants include (1) in situ measurements using selective electrodes (Gundersen et al., 1992; Archer et al., 1989; Wehrli et al., 1994); (2) in situ collection techniques using diffusive equilibrium thin films (DET) and diffusive gradient thin films (DGT) followed by laboratory analyses (Davison et al., 1991, 1994; Davison and Zhang, 1994; Zhang et al., 1995); and (3) equilibrium models (SOILCHEM) (Sposito and Coves, 1988).

Influence of Soil Amendments on Bioavailability

The removal of contaminated soil to mitigate exposure of terrestrial ecosystem components to Pb can often present both economic and logistical problems. Because of this, recent studies have focused on in situ methodologies to lower soil-Pb relative bioavailability (RBA) (Brown et al., 2003a,b). To date, the most common methods studied include the addition

of soil amendments in an effort to either lower the solubility of the Pb form or to provide sorption sites for fixation of pore-water Pb. These amendments typically fall within the categories of phosphate, biosolid, and Al/Fe/Mn-oxide amendments.

Phosphate amendments have been studied extensively and, in some cases, offer the most promising results (Brown et al., 1999; Ryan et al., 2001; Cotter-Howells and Caporn, 1996; Hettiarachchi et al., 2001, 2003; Rabinowitz, 1993; Yang et al., 2001; Ma et al., 1995). A number of potentially significant problems associated with phosphate amendments have been recognized. The added phosphate poses the potential risk of eutrophication of nearby waterways from soil runoff. There also may be both phyto- and earthworm toxicity (Ownby et al., 2005; Cao et al., 2002; Rusek and Marshall, 2000), primarily associated with very high applications of phosphorous and/or decreased soil pH. Indications of phytotoxicity are often balanced by studies such as Zhu et al. (2004) that illustrate a 50 to 70% reduction in shoot-root uptake of Pb in phosphate-amended soils. It also has been shown (Impellitteri, 2005; Smith et al., 2002; Chaney and Ryan, 1994; Ruby et al., 1994) that the addition of phosphate would enhance arsenic mobility (potentially moving arsenic down into the groundwater) through competitive anion exchange. Some data (Lenoble et al., 2005) indicate that this problem can be mitigated if arsenic and Pb contaminated soils could be amended with iron(III) phosphate, although there could still be issues with drinking water quality.

Biosolids have been used historically in the restoration of coal mines (Haering et al., 2000; Sopper, 1993). More recently, workers have demonstrated the feasibility of their use in the restoration of mine tailings (Brown et al., 2000) and urban soils (Brown et al., 2003a; Farfel et al., 2005). As with phosphate amendments, problems with biosolid application have also been documented. Studies have shown that metal transport is significantly accelerated in soils amended with biosolids (Al-Wabel et al., 2002; McBride et al., 1997, 1999; Lamy et al., 1993; Richards et al., 1998, 2000).

7.1.2 Distribution of Atmospherically Delivered Lead in Terrestrial Ecosystems

Advances in technology since the 1986 Lead AQCD have allowed for a more quantitative determination of the mobility, distribution, uptake, speciation, and fluxes of atmospherically-delivered Pb in terrestrial ecosystems. In most cases, Pb appears to be strongly bound to soils

and sediments in terrestrial ecosystems, which prevents substantial mobility and uptake in the terrestrial environment (e.g., Bacon et al., 2005). However, the controls on Pb speciation, and thus on mobility and potential bioavailability are not completely understood, so there remains a considerable need for more research on this topic.

Lead Speciation in Solid Phases

Lead can enter terrestrial ecosystems through natural rock weathering and by a variety of anthropogenic pathways. During the hydrolysis and oxidation of Pb-containing minerals, divalent Pb (Pb^{2+}) is released to the soil solution where it is rapidly fixed by organic matter and secondary mineral phases (Kabata-Pendias and Pendias, 1992; Erel et al., 1997). The geochemical form of natural Pb in terrestrial ecosystems will be strongly controlled by soil type, PH, and parent material (Emmanuel and Erel, 2002). In contrast, anthropogenically-introduced Pb has a variety of different geochemical forms, depending on the specific source. While Pb in soils from battery reclamation areas can be in the form of $PbSO_4$ or $PbSiO_3$, Pb in soils from shooting ranges and paint spills is commonly found as PbO and a variety of Pb carbonates (Vantelon et al., 2005; Laperche et al., 1996; Manceau et al., 1996). Atmospherically-delivered Pb resulting from fossil fuel combustion is typically introduced into terrestrial ecosystems as Pb-sulfur compounds and Pb oxides (Olson and Skogerboe, 1975; Clevenger et al., 1991; Batonneau et al., 2004; Utsunomiya et al., 2004). After deposition, Pb species are likely transformed. Although the specific factors that control the speciation of anthropogenic Pb speciation in soils are not well understood, there are many studies that have partitioned Pb into its different geochemical phases. A thorough understanding of Pb speciation is very important in order (a) to predict potential mobility and bioavailability and (b) to accurately apply a critical loads methodology for determining air quality standards (Lawlor and Tipping, 2003; Paces, 1998). See Section 7.3 for more discussion of critical loads methodology.

Selective chemical extractions have been employed extensively for quantifying amounts of a particular metal phase (e.g., PbS, Pb-humate, Pb-Fe, Mn oxide) present in soil rather than total metal concentration. Selective extractions can be a relatively rapid, simple, and inexpensive means for determining metal phases in soils, and the generated data can be linked to potential mobility and bioavailability of the metal (Tessier and Campbell, 1987). However, some problems persist with the selective extraction technique. First, extractions are rarely specific to a

single phase. For example, while peroxide (H_2O_2) is often used to remove metals bound in organic matter in soils, some researchers have demonstrated that this reagent destroys clay minerals and sulfides (Ryan et al., 2002). Peroxide solutions may also be inefficient at removing metals bound to humic acids and, in fact, could potentially result in the precipitation of metal-humate substances. In addition to non-selectivity of reagents, significant metal redistribution has been documented during sequential chemical extractions (Ho and Evans, 2000; Sulkowski and Hirner, 2006), and many reagents may not extract targeted phases completely. Therefore, while chemical extractions do provide some useful information on metal phases in soil and scenarios for mobilization, the results should be treated as "operationally defined," e.g., "H_2O_2 liberated-Pb" rather than "organic Pb."

Synchrotron radiation (X-rays) allows researchers to probe the electron configuration of metals in untreated soil samples. Because different elements have different electron binding energies, X-rays can be focused in an energy window specific to a metal of interest. The precise energy required to dislodge a core electron from a metal will be a function of the oxidation state and covalency of the metal. Since the electron configuration of a Pb atom is directly governed by its speciation (e.g., Pb bound to organics, Pb adsorbed to oxide surfaces, PbS, etc.), X-ray absorption experiments are a powerful in situ technique for determining speciation that does not suffer from some of the problems of chemical extractions (Bargar et al., 1997a,b; Bargar et al., 1998).

Selective chemical extractions and synchrotron-based X-ray studies have shown that industrial Pb can be strongly sequestered by organic matter and secondary minerals such as clays and oxides of Al, Fe, and Mn (Miller and McFee, 1983; Jersak et al., 1997; Johnson and Petras, 1998; Kaste et al., 2005). More recent X-ray studies have demonstrated the importance of biomineralization of Pb in soils by bacteria and nematodes (Jackson et al., 2005; Templeton et al., 2003a,b; Xia et al., 1997).

Lead Solid-Solution Partitioning

The concentration of Pb species dissolved in soil solution is probably controlled by some combination of a) Pb mineral solubility equilibria, b) adsorption reactions of dissolved Pb phases on inorganic surfaces (e.g., oxides of Al, Fe, Si, Mn, etc., clay minerals), and c) adsorption reactions of dissolved Pb phases on soil organic matter. Dissolved Pb phases in soil solution can

be some combination of Pb^{2+} and its hydrolysis species, Pb bound to dissolved organic matter, and Pb complexes with inorganic ligands such as Cl^- and SO_4^{2-}. Alkaline soils typically have solutions supersaturated with respect to $PbCO_3$, $Pb_3(CO_3)_2(OH)_2$, $Pb(OH)_2$, $Pb_3(PO_4)_2$, $Pb_5(PO_4)_3(OH)$, and $Pb_4O(PO_4)_2$ (Badawy et al., 2002). Pb phosphate minerals in particular, are very insoluble, and calculations based on thermodynamic data predict that these phases will control dissolved Pb in soil solution under a variety of conditions (Nriagu, 1974; Ruby et al., 1994). However, certain chelating agents, such as dissolved organic matter can prevent the precipitation of Pb minerals (Lang and Kaupenjohann, 2003), and the natural formation of these minerals has not yet been observed in terrestrial ecosystems (Kaste et al., 2006).

Increasing soil solution dissolved organic matter content and decreasing pH typically are strongly correlated with increases in the concentration of dissolved Pb species (Badawy et al., 2002; Sauvé et al., 1998, 2000a,b, 2003; Tipping et al., 2003; Weng et al., 2002). In the case of adsorption phenomena, the partitioning of Pb^{2+} to the solid phase is also controlled by total metal loading: high Pb loadings will result in a lower fraction partitioned to the solid phase. Sauvé et al. (1998; 1997) demonstrated that only a fraction of the total Pb in solution was actually Pb^{2+} in soils treated with leaf compost. The fraction of Pb^{2+} to total dissolved Pb ranged from <1 to 60%, depending on pH and the availability of Pb-binding ligands. In acidic soils, Al species can compete for sites on natural organic matter and inhibit Pb binding to surfaces (Gustafsson et al., 2003).

Tracing the Fate of Atmospherically Delivered Lead

Radiogenic Pb isotopes offer a powerful tool for separating anthropogenic Pb from natural Pb derived from mineral weathering (Erel and Patterson, 1994; Erel et al., 1997). This is particularly useful for studying Pb in mineral soil, where geogenic Pb often dominates. The ore bodies from which anthropogenic Pb are typically derived are usually enriched in ^{207}Pb relative to ^{206}Pb and ^{208}Pb when compared with Pb found in granitic rocks. Uranium-238 series ^{210}Pb also provides a tool for tracing atmospherically delivered Pb in soils. Fallout ^{210}Pb is deposited onto forests via wet and dry deposition, similar to anthropogenic Pb deposition in forests, and is thus useful as a tracer for non-native Pb in soils. ^{210}Pb is convenient to use for calculating the residence time of Pb in soil layers because its atmospheric and soil fluxes can be assumed to be in steady-state at undisturbed sites (Dörr, 1995; Dörr and Munnich, 1989; Kaste et al., 2003).

Researchers assessing the fate of atmospheric Pb in soils have also relied on repeated sampling of soils and vegetation for total Pb. This technique works best when anthropogenic Pb accounts for the vast majority of total Pb in a particular reservoir. Johnson et al. (1995), Yanai et al. (2004), and Friedland et al. (1992) used O horizon (forest floor) time series data to evaluate the movement of gasoline-derived Pb in the soil profile. Surface soils sampled relatively recently demonstrate that the upper soil horizons (O + A horizons) are retaining most of the anthropogenic Pb burden introduced to the systems during the 20th century (Evans et al., 2005). Miller and Friedland (1994) and Wang and Benoit (1997) suggested that the vertical movement of organic particles dominated Pb transport in the soil profile.

By describing the movement of atmospherically-delivered Pb in terrestrial ecosystems, we can begin to predict the Pb inventories of various ecosystem compartments that a particular atmospheric deposition rate will support. This type of information is very pertinent to air quality issues. For example, if the rate of Pb loss is known for a particular soil horizon and reasonable assumptions can be made about biogeochemical cycling and chemical weathering inputs, then steady-state Pb concentrations can be calculated for any constant deposition rate (in mass of Pb deposited per square meter). First-order rate loss constants, *k*, have been calculated for organic horizons using forest floor inventories, radiogenic ^{207}Pb tracer techniques, and fallout ^{210}Pb (Miller and Friedland, 1994; Johnson et al., 1995; Kaste et al., 2003; Watmough et al., 2004; Kaste et al., 2006). First order rate loss constants vary substantially, ranging between −0.003 to −0.6 (1/y), depending on soil type and climate. Wang and Benoit (1997) used the first-order rate loss technique to model forest floor Pb dynamics at the Hubbard Brook Experimental Forest in New Hampshire. They concluded that with steady Pb deposition at 0.0065 kg/ha/y, the forest floor would reach a steady-state Pb concentration of 1.4 ppm. Calculated steady-state Pb contents of different ecosystem compartments can then be compared with experimentally-derived toxicity thresholds (Liang and Tabatabai, 1977; 1978) to put deposition rates into context with the terrestrial ecosystem.

7.1.3 Species Response/Mode of Action

The current document expands upon and updates knowledge since 1986 related to the uptake, detoxification, physiological effects, and modifying factors of Pb toxicity to terrestrial

organisms. Terrestrial organisms discussed in this chapter include soil organisms, plants, birds, and mammals.

Uptake into Plants and Invertebrates

Recent work supports previous results and conclusions that surface deposition of Pb onto above-ground vegetation from airborne sources may be significant (Dalenberg and Van Driel, 1990; Jones and Johnston, 1991; Angelova et al., 2004). In addition, most Pb is taken up by plants via the symplastic route (through cell membranes) (Sieghardt, 1990) and remains in the roots, with little translocation to shoots, leaves, or other plant parts. Different species of plants and invertebrates accumulate different amounts of Pb (Pižl and Josens, 1995; Terhivuo et al., 1994; Wierzbicka, 1999).

Recent work supports previous conclusions that the form of metal tested, and its speciation in soil, influence uptake and toxicity to plants and invertebrates. The oxide form is less toxic than the chloride or acetate forms, which are less toxic than the nitrate form of Pb (Khan and Frankland, 1983; Lock and Janssen, 2002; Bongers et al., 2004). However, these results must be interpreted with caution, as the counter ion (e.g., the nitrate ion) may be contributing to the observed toxicity (Bongers et al., 2004).

Detoxification in Plants and Invertebrates

Lead may be deposited in root cell walls as a detoxification mechanism, and this may be influenced by calcium (Antosiewicz, 2005). Yang et al. (2000) suggested that the oxalate content in root and root exudates reduced the bioavailability of Pb in soil, and that this was an important tolerance mechanism. Other hypotheses put forward recently include the presence of sulfur ligands (Sharma et al., 2004) and the sequestration of Pb in old leaves (Szarek-Lukaszewska et al., 2004) as detoxification mechanisms.

Lead detoxification has not been studied extensively in invertebrates. Glutathione detoxification enzymes were measured in two species of spider (Wilczek et al., 2004). Lead may be stored in waste nodules in earthworms (Hopkin, 1989) or as pyromorphite in the nematode (Jackson et al., 2005).

Physiological Effects

The effects on heme synthesis (as measured by 5-aminolaevulinic acid dehydratase [ALAD] activity and protoporphyrin concentration, primarily) had been well-documented in the 1986 AQCD (U.S. Environmental Protection Agency, 1986) and continue to be studied (Schlick et al., 1983; Scheuhammer, 1989; Redig et al., 1991; Henny et al., 1991; Beyer et al., 2000; Hoffman et al., 2000a, b). However, Henny et al. (1991) caution that changes in ALAD and other enzyme parameters are not always related to adverse effects, but simply indicate exposure. Other effects on plasma enzymes, which may damage other organs, have been reported (Brar et al., 1997a, b). Lead also may cause lipid peroxidation (Mateo and Hoffman, 2001) which may be alleviated by Vitamin E, although lead poisoning may still result (Mateo et al., 2003b). Changes in fatty acid production have been reported, which may influence immune response and bone formation (Mateo et al., 2003a).

Response Modification

Genetics, biological factors, physical/environmental factors, nutritional factors and other pollutants can modify terrestrial organism response to Pb. Fisher 344 rats were found to be more sensitive to Pb than Sprague-Dawley rats (Dearth et al., 2004). Younger animals are more sensitive than older animals (Eisler, 1988; Scheuhammer, 1991), and females generally are more sensitive than males (Scheuhammer, 1987; Tejedor and Gonzalez, 1992; Snoeijs et al., 2005). Monogastric animals are more sensitive than ruminants (Humphreys, 1991). Insectivorous mammals may be more exposed to Pb than herbivores (Beyer et al., 1985; Sample et al., 1998), and higher tropic-level consumers may be less exposed than lower trophic-level organisms (Henny et al., 1991). Diets deficient in nutrients (including calcium) result in increased uptake of Pb (Snoeijs et al., 2005) and greater toxicity (Douglas-Stroebel et al., 2005) in birds, relative to diets containing adequate nutrient levels.

Mycorrhizal fungi may ameliorate Pb toxicity until a threshold is surpassed (Malcová and Gryndler, 2003), which may explain why some studies show increased uptake into plants (Lin et al., 2004) while others show no difference or less uptake (Dixon, 1988). Uptake of Pb into plants and soil invertebrates increases with a decrease in soil pH. However, calcium content, organic matter content, and cation exchange capacity of soils also had a significant influence on uptake of Pb into plants and invertebrates (Beyer et al., 1987; Morgan and Morgan, 1988).

Interactions of Pb with other metals are inconsistent, depending on the endpoint measured, the tissue analyzed, the animal species, and the metal combination (Phillips et al., 2003; An et al., 2004; Garcia and Corredor, 2004; He et al., 2004; Perottoni et al., 2005).

7.1.4 Exposure/Response of Terrestrial Species

The current document expands upon and updates knowledge related to the effects of Pb on terrestrial primary producers, consumers and decomposers found in the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986). Lead exposure may adversely affect organisms at different levels of organization, i.e., individual organisms, populations, communities, or ecosystems. Generally, however, there is insufficient information available for single materials in controlled studies to permit evaluation of specific impacts on higher levels of organization (beyond the individual organism). Potential effects at the population level or higher are, of necessity, extrapolated from individual level studies. Available population, community, or ecosystem level studies are typically conducted at sites that have been contaminated or adversely affected by multiple stressors (several chemicals alone or combined with physical or biological stressors). Therefore, the best documented links between Pb and effects on the environment are with effects on individual organisms. Impacts on terrestrial ecosystems are discussed in Section 7.1.5 and Annex AX7.1.5.

Primary Producers

Effects of Pb on terrestrial plants include decreased photosynthetic and transpiration rates, and decreased growth and yield. The phytotoxicity of Pb is considered to be relatively low, compared to other metals, and there are few reports of phytotoxicity from Pb exposure under field conditions. Phytotoxicity data recently were reviewed for the development of the ecological soil screening levels (Eco-SSL) (U.S. Environmental Protection Agency, 2005b). Many of the toxicity data presented in U.S. Environmental Protection Agency (2005b) are lower (i.e., they represent greater toxicity) than those discussed in the 1986 Lead AQCD, although both documents acknowledge that toxicity is observed over a wide range of Pb concentrations in soil (tens to thousands of mg/kg soil). This may be due to many factors, such as the soil conditions (e.g., pH, organic matter) and differences in bioavailability of the Pb in spiked soils, perhaps due to lack of equilibration of the Pb solution with the soil after spiking. Most phytotoxicity data

continue to be developed for agricultural plant species (i.e., vegetable and grain crops). Few data are available for trees or native herbaceous plants, although two of the five ecotoxicological endpoints used to develop the Eco-SSL were for trees and two were for clover.

Consumers

Effects of Pb on avian and mammalian consumers include decreased survival, reproduction, and growth, as well as effects on development and behavior. There remain few field effects data for consumers, except from sites with multiple contaminants, for which it is difficult to attribute toxicity specifically to Pb. Avian and mammalian toxicity data recently were reviewed for the development of Eco-SSLs (U.S. Environmental Protection Agency, 2005b). Many of the toxicity data presented by EPA (U.S. Environmental Protection Agency, 2005b) are lower than those discussed in the 1986 Lead AQCD, (i.e., the Eco-SSL document describes studies which report greater toxicity of Pb to various organisms), although EPA (U.S. Environmental Protection Agency, 2005b) recognizes that toxicity is observed over a wide range of doses (<1 to >1,000 mg Pb/kg bw-day). Most toxicity data for birds are derived from chicken and quail studies, and most data for mammals are derived from laboratory rat and mouse studies. Data derived for other species would contribute to the understanding of Pb toxicity, particularly for wildlife species with different gut physiologies. In addition, data derived using environmentally-realistic exposures, such as from Pb-contaminated soil and food may be recommended. Finally, data derived from inhalation exposures, which evaluate endpoints such as survival, growth, and reproduction, would contribute to understanding the implications of airborne releases of Pb.

Decomposers

Effects of Pb on soil invertebrates include decreased survival, growth and reproduction. Effects on microorganisms include changes in nitrogen mineralization, and changes in enzyme activities. Recent data on Pb toxicity to soil invertebrates and microorganisms are consistent with those reported in the 1986 Lead AQCD, with toxicity generally being observed at concentrations of hundreds to thousands of mg Pb/kg soil. Studies on microbial processes may be influenced significantly by soil parameters, and the significance of the test results is not clear.

Ecological Soil Screening Levels (Eco-SSLs)

Eco-SSLs are concentrations of contaminants in soils that would result in little or no measurable effect on ecological receptors (U.S. Environmental Protection Agency, 2005a). They were developed by U.S. EPA for use in the screening-level assessments at Superfund sites to identify those contaminants needing further investigation, and also to identify those contaminants that are not of potential ecological concern and do not need to be considered in the subsequent analyses. They were developed following rigorous scientific protocols, and were subjected to two rounds of peer review. However, several conservative factors were incorporated into their development. For the plant and invertebrate Eco-SSLs, studies were scored to favor relatively high bioavailability. For wildlife Eco-SSLs, only species with a clear exposure link to soil were considered (generalist species, species with a link to the aquatic environment, or species which consume aerial insects were excluded), simple diet classifications were used (100% plants, 100% earthworms or 100% animal prey) when in reality wildlife consume a varied diet, species were assumed to forage exclusively at the contaminated site, relative bioavailability or Pb in soil and diet was assumed to be 1, and the TRV was selected as the geometric mean of NOAELs unless this value was higher than the lowest bounded LOAEL for mortality, growth or reproduction (U.S. Environmental Protection Agency, 2005a,b). The Eco-SSLs are intentionally conservative in order to provide confidence that contaminants which could present an unacceptable risk are not screened out early in the evaluation process. That is, at or below these levels, adverse effects are considered unlikely. Due to conservative modeling assumptions (e.g., metal exists in most toxic form or highly bioavailable form, high food ingestion rate, high soil ingestion rate), which are common to screening processes, several Eco-SSLs are derived below the average background soil concentration for a particular contaminant. For example, Scheuhammer et al. (2003) found that ninety-one percent (64/70) of the soil samples analyzed in eastern Canada had <45 mg/kg Pb, concentrations typical of noncontaminated rural soils in Canada and elsewhere in North America (Breckenridge and Crockett, 1998; McKeague and Wolynetz, 1980). However, the Eco-SSL for birds (based on the American woodcock) is recommended as 11 mg/kg (U.S. Environmental Protection Agency, 2005a,b).

The Eco-SSLs for terrestrial plants, birds, mammals, and soil invertebrates are 120 mg/kg, 11 mg/kg, 56 mg/kg and 1700 mg/kg, respectively. See Annex Section AX7.1.4 for additional information.

7.1.5 Effects of Lead on Natural Terrestrial Ecosystems

Few significant effects of Pb pollution have been observed at sites that are not near point sources of Pb. At present, industrial point sources such as smelter sites represent the greatest Pb-related threat to the maintenance of sustainable, healthy, diverse, and high-functioning terrestrial ecosystems in the United States. However, assessing the risks specifically associated with Pb is difficult because these sites also experience elevated concentrations of other metals and because of effects related to SO_2 emissions. Terrestrial ecosystems may respond to stress in a variety of ways, including reductions in the vigor and/or growth of vegetation, reductions in biodiversity, and effects on energy flow and biogeochemical cycling.

Influence of Acidification

Like most metals, the solubility of Pb increases as pH decreases (Stumm and Morgan, 1995), suggesting that enhanced mobility of Pb should be found in ecosystems under acidification stress. However, Pb is also strongly bound to organic matter in soils and sediments. Reductions in pH may cause a decrease in the solubility of dissolved organic matter (DOM), due to the protonation of carboxylic functional groups (Tipping and Woof, 1990). Because of the importance of Pb complexation with organic matter, lower DOM concentrations in soil solution resulting from acidification may offset the increased solubility of Pb and hence decrease the mobility of the organically bound metal. Increased mobility was only observed in very acidic soils, those with pH <4.5 (Blake and Goulding, 2002). Acidification also may enhance Pb export to drainage water in very sandy soils, with limited ability to retain organic matter (Swanson and Johnson, 1980; Turner et al., 1985).

Influence of Land Use and Industry

Changes in land use represent potentially significant changes in the cycling of organic matter in terrestrial ecosystems. Conversion of pasture and croplands to woodlands changes the nature and quantity of organic matter inputs to the soil. The introduction of industrial activity may have consequences for organic matter cycling, and subsequently, Pb mobilization. In a rare long-term study of polluted soils, Egli et al. (1999) found that loss of soil carbon can induce the mobilization and loss of Pb from terrestrial ecosystems. However, it is worth noting that the decline in soil Pb was considerably smaller than the decline in organic carbon. This suggests

that Pb mobilized during organic matter decomposition can resorb to remaining organic matter or perhaps to alternate binding sites (e.g., Fe and Mn oxides).

Forest harvesting represents a severe disruption of the organic matter cycle in forest ecosystems. However, observations from clear-cut sites in the United States and Europe indicate that forest harvesting causes little or no mobilization or loss of Pb from forest soils (Berthelsen and Steinnes, 1995; Fuller et al., 1988). The principal risk associated with forest harvesting is the loss of Pb in particulate form to drainage waters through erosion.

Effects Observed Around Industrial Point Sources

The effects of Pb exposure on natural ecosystems are confounded by the fact that Pb exposure cannot be decoupled from other factors that may also affect the ecosystem under consideration. Principal among these factors are other trace metals and acidic deposition. Emissions of Pb from smelting and other industrial activities are accompanied by other trace metals (e.g., Zn, Cu, Cd) and sulfur dioxide (SO_2) that may cause toxic effects independently or in concert with Pb.

Natural terrestrial ecosystems near smelters, mines, and other industrial plants have exhibited a variety of effects related to ecosystem structure and function. These effects include decreases in species diversity, changes in floral and faunal community composition, and decreasing vigor of terrestrial vegetation. All of these effects were observed in ecosystems surrounding the Anaconda copper smelter in southwestern Montana, which operated between 1884 and 1980 (Galbraith et al., 1995; Kapustka et al., 1995). Similar observations were made in the area surrounding Palmerton, Pennsylvania, where two zinc smelters operated between 1898 and 1980 (Jordan, 1975; Sopper, 1989; Storm et al., 1994). Subsequent to the effects on vegetation, wind and erosion may remove litter and humus, leaving bare mineral soil, a nearly sterile environment in which very little energy transfer takes place (Little and Martin, 1972; Galbraith et al., 1995). Metal pollution around a Pb-Zn smelter near Bristol, England has not resulted in the loss of oak woodlands within 3 km of the smelter, despite significant accumulation of Pb, Cd, Cu, and Zn in soils and vegetation (Martin and Bullock, 1994). However, the high metal concentrations have favored the growth of metal-tolerant species in the woodland.

The effects of Pb and other chemical emissions on terrestrial ecosystems near smelters and other industrial sites decrease downwind from the source. Several studies using the soil burden as an indicator have shown that much of the contamination occurs within a radius of 20 to 50 km around the emission source (e.g., Miller and McFee, 1983; Martin and Bullock, 1994; Galbraith et al., 1995; Spurgeon and Hopkin, 1996). Elevated metal concentrations around smelters have been found to persist despite significant reductions in emissions (Hrsak et al., 2000). The confounding effect of other pollutants makes the assessment of Pb-specific exposure-response relationships very difficult at the whole-ecosystem level.

Influence of Climate Change

Atmospheric Pb is not likely to contribute significantly to global climate change. The potential linkages between climate-related stress and Pb cycling are poorly understood. Effects related to alterations in organic matter cycling may influence Pb migration. For example, an increase in temperature leading to increased rates of organic matter decomposition could lead to temporary increases in DOM concentrations and smaller steady-state pools of soil organic matter. There also is some evidence for recent increases in the frequency of soil freezing events in the northeastern United States (Mitchell et al., 1996). Soil freezing occurs when soils have little or no snow cover to insulate them from cold temperatures and results in an increased release of nitrate and DOC from the O horizons of forest soils (Mitchell et al., 1996; Fitzhugh et al., 2001). Increased fluctuations in precipitation may induce more frequent flooding, potentially increasing inputs of Pb and other metals to floodplain soils (Krüger and Grongroft, 2004). All of these factors could result in increased concentrations of Pb in waters draining terrestrial ecosystems.

Influence on Energy Flow and Biogeochemical Cycling

Lead can have a significant effect on energy flow in terrestrial ecosystems. In terrestrial ecosystems, energy flow is closely linked to the carbon cycle. The principal input of energy to terrestrial ecosystems is through photosynthesis, in which CO_2 is converted to biomass carbon. Because of this link between photosynthesis and energy flow, any effect that Pb has on the structure and function of terrestrial ecosystems influences the flow of energy into the ecosystem.

At some sites severely affected by metal pollution, death of vegetation can occur, dramatically reducing the input of carbon to the ecosystem (Jordan, 1975; Galbraith et al., 1995).

Lead influences energy transfer within terrestrial ecosystems, which begins with the decomposition of litter and other detrital material by soil bacteria and fungi, and cascades through the various components of the detrital food web. In acid- and metal-contaminated soils or soils treated with Pb investigators have documented significant declines in litter decomposition rates (Cotrufo et al., 1995; Johnson and Hale, 2004) and/or the rate of carbon respiration (Laskowski et al., 1994; Cotrufo et al., 1995; Saviozzi et al., 1997; Niklínska et al., 1998; Palmborg et al., 1998; Aka and Darici, 2004). The resulting accumulation of organic matter on the soil surface can be dramatic.

Because Pb mobility in soils is closely tied to organic matter cycling, decomposition processes are central to the biogeochemical cycle of Pb. Reduced decomposition rates in polluted ecosystems are the result of the inhibition of soil bacteria and fungi and its effects on microbial community structure (Bååth, 1989). Lead and other metals also inhibit the mineralization of nitrogen from soil organic matter and nitrification (Liang and Tabatabai, 1977, 1978; Senwo and Tabatabai, 1999; Acosta-Martinez and Tabatabai, 2000; Ekenler and Tabatabai, 2002), resulting in lower nitrogen availability to plants. This suggests that the inhibitory effect of Pb and other metals is broad-based, and not specific to any particular metabolic pathway. It is important to note that terrestrial sites that have exhibited significant disruption to energy flows and C processing are sites that have experienced severe metal contamination from smelters or other metals-related activities.

7.2 AQUATIC ECOSYSTEMS

The overall intent of this Section 7.2 is to provide sufficient information to support development of air quality criteria for Pb that is protective of aquatic ecosystems. To achieve this objective, the logical starting points are to (1) gain a general understanding of the current distribution and concentrations of Pb in the aquatic environment and (2) identify the threshold levels for Pb effects on aquatic populations, communities, and ecosystems. Ambient water quality criteria for Pb and other chemicals represent surface water concentrations intended to be protective of aquatic communities, including recreationally and commercially important species.

The EPA derives Ambient Water Quality Criteria (AWQC) to provide guidance to States and Tribes that are authorized to establish water quality standards under the Clean Water Act (CWA). Similarly, EPA has recommended sediment quality benchmarks for Pb and other divalent metals that, although not truly being criteria, do represent concentrations in sediment that are derived to be protective of benthic (sediment) organisms. As summarized further below and in subsequent sections, the U.S. EPA has increasingly focused on developing AWQC and sediment quality benchmarks for Pb and other metals that account for the bioavailability of the metal to aquatic life. These criteria and benchmark concentrations in water and sediment represent appropriate starting points to ensure that air quality criteria for Pb are adequately protective of aquatic life.

Since publication of the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986), knowledge has expanded with regard to the fate and effects of Pb in aquatic ecosystems and on the distribution and concentrations of Pb in surface waters throughout the United States. In addition, chemical, physical, and biological properties of Pb are discussed. The following provides a general overview of the key information found in corresponding Annex sections (Sections AX7.2.1 through AX7.2.5).

7.2.1 Methodologies Used in Aquatic Ecosystem Research

Ambient Water Quality Criteria and Bioavailability

The U.S. EPA guidelines for developing AWQC (Stephan et al., 1985) were published more than 20 years ago. Scientific advances in aquatic toxicology and risk assessment have been made since the 1980s. For example, the toxicological importance of dietary metals has been increasingly recognized and approaches for incorporating dietary metals into regulatory criteria are being evaluated (Meyer et al., 2005). Other issues include consideration of certain sublethal endpoints that are currently not directly incorporated into AWQC development (e.g., endocrine toxicity, behavioral responses) and protection of threatened and endangered (T&E) species (U.S. Environmental Protection Agency, 2003). In deriving appropriate and scientifically defensible air quality criteria for Pb, it will be important that the state-of-the-science for metals toxicity in aquatic systems be considered in the development process.

The primary form of Pb in freshwater and marine environments is divalent Pb (Pb^{2+}). In surface waters, the bioavailability of Pb to aquatic biota is driven by a variety of factors,

including calcium, dissolved organic carbon (DOC), pH, alkalinity, and total suspended solids (TSS). Accounting for the influence of calcium and magnesium ions on Pb bioavailability, the current AWQC for Pb are normalized to the hardness of the receiving water (Table 7-1).

Table 7-1. Summary of Lead Ambient Water Quality Criteria for Freshwater Organisms at Different Hardness Levels[1] (criteria expressed as dissolved lead)

Hardness (mg/L as CaCO3)	Acute Criterion (µg/L)	Chronic Criterion (µg/L)
50	30	1.2
100	65	2.5
200	136	5.3

[1] The acute and chronic criteria values are based on empirical data on the relationship between toxicity and hardness.

More recently, the biotic ligand model (BLM), which considers the binding of free metal ion to the site of toxic action and competition between metal species and other ions, has been developed to predict the toxicity of several metals under a variety of water quality conditions. However, there are limitations to this tool in deriving AWQC because, currently, only limited work has been conducted in developing chronic BLMs (for any metals, let alone Pb) and the acute BLMs to-date do not account for dietary metal exposures.

The U.S. EPA is currently revising the aquatic life AWQC for Pb, which will include toxicity data published after the 1985 AWQC were released and incorporation of the BLM is being evaluated.

Sediment Quality Benchmarks and Bioavailability

As in surface waters, there are a number of factors in sediment that can influence Pb bioavailability to benthic (sediment) organisms. Although sediment quality criteria have not been formally adopted, the EPA has published an equilibrium partitioning procedure for developing sediment criteria for metals (U.S. Environmental Protection Agency 2005c).

Equilibrium partitioning (EqP) theory predicts that metals partition in sediment between acid volatile sulfide, pore water, benthic organisms, and other sediment phases, such as organic carbon. When the sum of the molar concentrations of simultaneously extracted metal (∑SEM) minus the molar concentration of AVS is less than zero, it can accurately be predicted that sediments are not toxic because of these metals. Further, if ∑SEM-AVS is normalized to the fraction of organic carbon (i.e., (∑SEM-AVS)/fOC), mortality can be more reliably predicted by accounting for both the site-specific organic carbon and AVS concentrations (Table 7-2).

Table 7-2. Summary of Sediment Quality Benchmarks and Guidelines for Lead

Benchmark/ Guideline Type	Source	Effect Level	Value
Equilibrium partitioning	U.S. Environmental Protection Agency (2005c)	Low risk of adverse biological effects	(SEM-AVS)/f_{OC} < 130 µmol/g_{OC}
		May have adverse biological effects	130 µmol/g_{OC} < (SEM-AVS)/f_{OC} < 3,000 µmol/g_{OC}
		Adverse biological effects expected	(SEM-AVS)/f_{OC} > 3,000 µmol/g_{OC}
Bulk sediment	MacDonald et al. (2000)	TEC	35.8 µg/g dry wt.
		PEC	128 µg/g dry wt.
	Ingersoll et al. (1996)	ERL	55 µg/g dry wt.
		ERM	99 µg/g dry wt.
	Long et al. (1995)	ERL	46.7 µg/g dry wt.
		ERM	218 µg/g dry wt.

AVS = Acid volatile sulfide; ERL = Effects range – low (sediment concentration below which adverse effects are rarely observed or predicted among sensitive species, Long et al. [1995]); ERM = Effects range – median (sediment concentration above which effects are frequently or always observed or predicted among most species, Long et al. [1995]); oc = Organic carbon (f_{OC} = fraction organic carbon, g_{OC} = grams organic carbon); PEC = Probably effect concentration (sediment concentration above which harmful effects are likely to be observed, MacDonald et al. [2000]); SEM = Simultaneously extracted metal; TEC = Threshold effect concentration (sediment concentration below which harmful effects are unlikely to be observed, MacDonald et al. [2000]).

An alternative approach for developing sediment quality guidelines is to use empirical correlations between metal concentrations in bulk sediment to associated biological effects, based on sediment toxicity tests (Table 7-2). These guidelines are based on total metal concentrations in sediment and do not account for the bioavailability of metals between sediments.

It should be noted that although EPA is favoring the AVS-SEM approach for regulating metals in sediments, there is not scientific consensus on this issue. Various studies suggest that ingestion of sediment particles by benthic organisms is an important exposure route not accounted for by AVS-SEM (e.g., Lee et al., 2000; Griscom et al., 2002) or that the AVS-SEM approach may not be the most accurate approach available for predicting non-toxic and toxic results in laboratory studies.

7.2.2 Distribution of Lead in Aquatic Ecosystems

Speciation of Lead in Aquatic Ecosystems

The speciation of Pb in the aquatic environment is controlled by many factors, such as, pH, salinity, sorption, and biotransformation processes. Lead is typically present in acidic aquatic environments as $PbSO_4$, $PbCl_4$, ionic Pb, cationic forms of Pb hydroxide, and ordinary hydroxide $Pb(OH)_2$. In alkaline, waters common species of Pb include anionic forms of Pb carbonate $Pb(CO_3)$ and hydroxide $Pb(OH)_2$. In freshwaters, Pb typically forms strong complexes with inorganic OH^- and CO_3^{2-} and weak complexes with Cl^- (Bodek et al., 1988; Long and Angino, 1977). The primary form of Pb in freshwaters at low pH (≤ 6.5) is predominantly Pb^{2+} and less abundant inorganic forms include $Pb(HCO)_3$, $Pb(SO_4)_2^{2-}$, PbCl, $PbCO_3$, and $Pb_2(OH)_2CO_3$. At higher pH (≥ 7.5) Pb forms hydroxide complexes ($PbOH^+$, $Pb(OH)_2$, $Pb(OH)_3^-$, $Pb(OH)_4^{2-}$). Lead speciation in seawater is a function of chloride concentration and the primary species are $PbCl^{3-} > PbCO_3 > PbCl_2 > PbCl^+ >$ and $Pb(OH)^+$ (Fernando, 1995).

Lead sorption to suspended or bed sediments or suspended organic matter typically increases with increasing pH, increasing amounts of iron or manganese; and with the polarity of particulate matter (e.g., clays). Adsorption decreases with water hardness (Syracuse Research Corporation [SRC], 1999). At higher pH, Pb precipitates as $Pb(OH)^+$ and $PbHCO_3^+$ into bed sediments (Weber, 1993). Conversely, at low pH, Pb is negatively sorbed (repelled from the adsorbent surface) (U.S. Environmental Protection Agency, 1979; Gao et al., 2003). In addition,

Pb may be remobilized from sediment due to a decrease in metal concentration in the solution phase, complexation with chelating agents (e.g., EDTA), and changing redox conditions (Gao et al., 2003). Changes in water chemistry (e.g., reduced pH or ionic composition) can cause sediment Pb to become re-mobilized and potentially bioavailable to aquatic organisms (Weber, 1993). Methylation may result in Pb's remobilization and reintroduction into the aqueous environmental compartment and its subsequent release into the atmosphere (SRC, 1999). However, methylation is not a significant environmental pathway controlling Pb fate in the aquatic environment.

Lead Concentrations in United States Surface Waters

Nationwide, data for Pb in surface waters, from 1991 onward, were compiled using the United States Geological Survey's (USGS) National Water-Quality Assessment (NAWQA) database. Data were compiled from locations categorized as "ambient" or "natural." Ambient refers to data collected from all sampling locations, while natural refers to data collected from sampling locations categorized as forest, rangeland, or reference. Summary statistics for surface water, sediment (bulk, <63 µm), and fish tissue (whole body and liver) are summarized in Table 7-3. Overall atmospheric sources of Pb are generally decreasing as regulations have removed Pb from gasoline and other products (Eisenreich et al., 1986); however, elevated Pb concentrations remain at sites near ongoing sources, such as near mining wastes or wastewater effluents.

Lead concentrations in lakes and oceans were generally found to be much lower than those measured in the lotic waters assessed by NAWQA. Surface water concentrations of dissolved Pb measured in Hall Lake, Washington in 1990 ranged from 2.1 to 1015.3 ng/L (Balistrieri et al., 1994) . Nriagu et al., 1996 found that the average surface water dissolved Pb concentrations measured in the Great Lakes (Superior, Erie, and Ontario) between 1991 and 1993 were 3.2, 6.0, and 9.9 ng/L, respectively. Pb concentrations ranged from 3.2 to 11 ng/L across all three lakes. Similarly, 101 surface water total Pb concentrations measured at the Hawaii Ocean Time-series (HOT) station ALOHA between 1998 and 2002 ranged from 25 to 57 pmol/kg (5 to 11 ng/kg; (Boyle et al., 2005). Based on the fact that Pb is predominately found in the dissolved form in the open ocean (<90%; Schaule and Patterson, 1981), dissolved Pb concentrations measured at these locations would likely have been even lower than the total Pb concentrations reported.

Table 7-3. Summary of Lead Concentrations in United States Surface Water, Sediment, and Fish Tissue

	Surface Water – Dissolved (µg/L)		Sediment – Bulk, <63 µm (µg/g dry wt.)		Fish Tissue (µg/g dry wt.)			
					Whole Organism		Liver	
Statistic	**Ambient**	**Natural**	**Ambient**	**Natural**	**Ambient**	**Natural**	**Ambient**	**Natural**
n	3,445	430	1,466	258	332	93	559	83
%ND	86	88	0.48	1.2	39	51	71	89
Min	0.04	0.04	0.50	0.50	0.08	0.08	0.01	0.01
Mean	0.66	0.52	120	109	1.03	0.95	0.36	0.28
Median	0.50	0.50	28	22	0.59	0.35	0.15	0.11
90th %ile	0.50	0.50	120	66	2.27	1.40	0.59	0.37
95th %ile	1.10	0.50	200	162	3.24	2.50	1.06	1.26
Max	29.78	8.40	12,000	12,000	22.6	22.6	12.7	3.37

%ND = Percentage not detected.

In open waters of the North Atlantic the decline of Pb concentrations has been associated with the phasing out of leaded gasoline in North America and western Europe (Véron et al., 1998). Likewise, restrictions reducing Pb in gasoline appear to have been effective in reducing atmospheric Pb loading to the Okefenokee Swamp in southern Georgia/northern Florida (Jackson et al., 2004). Based on sediment cores from the Okefenokee Swamp, Pb concentrations were ~0.5 mg/kg prior to industrial development, reached a maximum of ~31 mg/kg from about 1935 to 1965, and following passage of the Clean Air Act in 1970 concentrations declined to about 18 mg/kg in 1990 (Jackson et al., 2004). However, in estuarine systems, it appears that similar declines following the phase-out of leaded gasoline are not necessarily as rapid. Steding et al. (2000) used isotopic evidence to demonstrate the continued cycling of Pb in the San Francisco Bay estuary. In the southern arm of San Francisco Bay, which has an average depth of <2 m, Steding et al. (2000) found that isotopic compositions were essentially invariant, with 90% of the Pb derived from 1960s-1970s leaded gasoline. The authors attributed this to the limited hydraulic flushing and remobilization of Pb from bottom sediments. In the northern arm of San Francisco Bay, although seasonal and decadal variations in Pb isotope composition were observed, mass balance calculations indicate that only a small fraction of leaded gasoline fallout

from the late 1980s had been washed out of the San Joaquin and Sacramento rivers' drainage basin by 1995 and, consequently, freshwater inputs remain a Pb source to the bay (Steding et al., 2000). The authors suggest that the continuous source of Pb from the river systems draining into the bay, coupled with benthic remobilization of Pb, indicates that historic gasoline deposits may remain in the combined riparian/estuarine system for decades.

In addition to directly measuring Pb concentrations in various aquatic compartments, it is useful to study the vertical distribution of Pb. Sediment profiling and core dating is a method used to determine the extent of accumulation of atmospheric Pb and provides information on potential anthropogenic sources. Sediment concentration profiles are typically coupled with Pb isotopic analysis. The isotope fingerprinting method utilizes measurements of the abundance of common Pb isotopes (^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb) to distinguish between natural Pb over geologic time and potential anthropogenic sources. Studies of sediment profiles have suggested that observed increases in Pb concentrations in the upper sediment layer are concomitant with increases in anthropogenic inputs (Bloom and Crecelius, 1987; Case et al., 1989; Ritson et al., 1999; Chillrud et al., 2003). Isotopic ratios have been used to link increases in sediment concentrations with specific anthropogenic sources and to estimate historic records of Pb fluxes to surface waters and sediments (Flegal et al., 1987, 1989; Blais, 1996; Bindler et al., 1999). For example, Gallon et al. (2006) collected sediment cores from Canadian Shield headwater lakes along a 300 km transect extending from a nonferrous metal smelter and used ^{206}Pb/^{207}Pb ratios to differentiate Pb contributions from smelter emissions relative to Pb contributions from other anthropogenic inputs. The ^{206}Pb/^{207}Pb ratio for smelter emissions was 0.993, compared to ratios on the order of 1.15 to 1.22 in aerosols collected at sites remote from point sources in Eastern Canada and the United States. Based on these isotopic signatures, Gallon et al. (2006) were able to estimate the amounts of smelter-derived Pb in sediment collected along the 300-km transect.

7.2.3 Species Response/Mode of Action

Lead Uptake

Lead can bioaccumulate in the tissues of aquatic organisms through ingestion of food and water, and adsorption from water, and can subsequently lead to adverse effects if tissue levels are sufficiently high (Vink, 2002; Rainbow, 1996). The accumulation of Pb is influenced by pH and decreasing pH favors bioavailability and bioaccumulation. Bioconcentration factors (BCFs)

have been reported in the scientific literature for various organisms and range from 840 to 20,000 (aquatic plants), 499 to 3,670 (aquatic invertebrates), and 42 to 45 (fish). Organisms that bioaccumulate Pb with little excretion must partition the metal such that it has limited bioavailability, otherwise toxicity will occur if a sufficiently high concentration is reached.

Resistance Mechanisms

Aquatic organisms have various methods to resist the toxic effects of metals such as Pb. Resistance processes include detoxification and avoidance responses. Mechanisms of resistance and detoxification vary among aquatic biota. These processes can include translocation, excretion, chelation, adsorption, and vacuolar storage and deposition. For example, protists and plants produce intracellular polypeptides that form complexes with Pb (Zenk, 1996; Morelli and Scarano, 2001). Some macrophytes and wetland plants have developed translocation strategies for tolerance and detoxification (Knowlton et al., 1983; Deng et al., 2004). Various aquatic invertebrates may sequester Pb in the exoskeleton (Boisson et al., 2002; Knowlton et al., 1983) or have developed specialized excretion processes (Vogt and Quinitio, 1994). Fish scales and mucous may chelate Pb in the water column and potentially reduce Pb uptake (Coello and Khan, 1996).

Avoidance responses are actions performed to evade a perceived threat. Some aquatic organisms have been shown to be quite adept at avoiding Pb in aquatic systems, while others seem incapable of detecting its presence. Snails have been shown to be sensitive to Pb, and avoid it at high concentrations (Lefcort et al., 2004). Conversely, anuran (frog and toad) species lack an avoidance response up to 1000 µg Pb/L (Steele et al., 1991). Fish avoidance of chemical toxicants has been well established, and is a dominant sublethal response in polluted waters (Svecevičius, 2001). However, studies examining avoidance behavior of Pb in fish are lacking. In addition to the presence of toxic metals, light and pH can also alter preference-avoidance responses.

Physiological Effects of Lead

Physiological effects of Pb on aquatic biota can occur at the biochemical, cellular and tissue levels of organization. Lead has been shown to affect brain receptors in fish (Rademacher et al., 2005) and serum enzyme activity (e.g., EROD and ALAD) in fish and amphibians (Kutlu

and Susuz, 2004; Blasco and Puppo, 1999; Gill et al., 1991; Vogiatzis and Loumbourdis, 1999). Studies examining the effects of Pb on fish blood chemistry have indicated alterations from acute and chronic exposures ranging from 100 to 10,000 µg/L (Gill et al., 1991; Allen, 1993; Gopal et al., 1997). Lead exposure has also been shown to negatively affect the growth of aquatic invertebrates (Arai et al., 2002).

Factors that Modify Organism Response to Lead

There are several factors that may influence organism response to Pb exposure. These may include the size or age of an organism, genetics, environmental factors (e.g., pH, salinity), nutrition, and the presence of other contaminants. Lead accumulation in living organisms is controlled, in part, by metabolic rates (Farkas et al., 2003) and by the physiological conditions of an organism. Relationships between age, size and Pb body burden in aquatic invertebrates and fish are variable and depend on many environmental variables (e.g., exposure) (Farkas et al., 2003). For example, examination of Pb exposure (up to 100 µg/L) in aquatic invertebrates showed little relationship between body size and Pb accumulation (MacLean et al., 1996; Canli and Furness, 1993), whereas Pb accumulation and fish size were found to be positively correlated (Douben, 1989; Köck et al., 1996).

The genetics of an organism and/or population may alter the response to Pb exposure through one of two processes: (1) a contaminant may influence selection, by selecting for certain phenotypes that enable populations to better cope with the chemical, or (2) a contaminant can be genotoxic, meaning it can produce alterations in nucleic acids at sublethal exposure levels, resulting in changes in hereditary characteristics or DNA inactivation (Shugart, 1995). Genetic selection has been observed in aquatic organisms due to Pb tolerance. Because tolerant individuals have a selective advantage over vulnerable individuals in polluted environments, the frequency of tolerance genes will increase in exposed populations over time (Beaty et al., 1998). Several studies have shown that heavy metals can alter population gene pools resulting in decreased genetic diversity (Duan et al., 2000; Kim et al., 2003). Laboratory studies have shown that exposure to Pb at 10 mg Pb^{2+}/mL of blood leads to chromosomal aberrations in some aquatic organisms (Cestari et al., 2004). Lead exposure in water (50 µg/L) over four weeks resulted in DNA strand breakage in the freshwater mussel *Anodonta grandis* (Black et al., 1996). More recently, Cestari et al. (2004) observed similar results (increase in the frequency of chromosomal

aberrations and DNA damage in kidney cell cultures) in fish (*Hoplias malabaricus*) that were fed Pb contaminated food over 18, 41 and 64 days.

Environmental factors can alter the availability, uptake and toxicity of Pb to aquatic organisms. Van Hattum et al. (1996) studied the influence of abiotic variables, including dissolved organic carbon (DOC) on Pb concentrations in freshwater isopods and found that as DOC concentrations increased, BCFs decreased in *P. meridianus* and *A. aquaticus*, indicating that DOC acts to inhibit the availability of Pb to these isopods. Schwartz et al. (2004) collected natural organic matter (NOM) from several aquatic sites across Canada and investigated the effects of NOM on Pb toxicity in rainbow trout (*Oncorhynchus mykiss*). The results showed that NOM in test water almost always increased LT_{50} (time to reach 50% mortality), and optically dark NOM tended to decrease Pb toxicity more than did optically light NOM in rainbow trout. Studies generally agree that the toxicity of Pb decreases as pH increases (MacDonald et al., 2002; Horne and Dunson, 1995a,b,c). As pH decreases, Pb becomes more soluble and more readily bioavailable to aquatic organisms (Weber, 1993). Acute and chronic toxicity of Pb increases with decreasing water hardness, as Pb becomes more soluble and bioavailable to aquatic organisms (Horne and Dunson, 1995c; Borgmann et al., 2005). There is some evidence that water hardness and pH work together to increase or decrease the toxicity of Pb. High Ca^{2+} concentrations have been shown to protect against the toxic effects of Pb (Sayer et al., 1989; Rogers and Wood, 2004; MacDonald et al., 2002; Hassler et al., 2004). Ca^{2+} affects the permeability and integrity of cell membranes and intracellular contents (Sayer et al., 1989). As Ca^{2+} concentrations decrease, the passive flux of ions (e.g., Pb) and water increases. Finally, increasing salinity was found to decrease Pb toxicity (Verslycke et al., 2003). The reduction in toxicity was attributed to increased complexation of Pb^{2+} with Cl^{-} ions.

Nutrients (e.g., nitrate, carbonate) have been shown to affect Pb toxicity in some aquatic organisms. Jampani (1988) looked at the impact of various nutrients (i.e., sodium acetate, citric acid, sodium carbonate, nitrogen, and phosphates) on reducing growth inhibition in blue-green algae (*Synechococcus aeruginosus*) exposed to 200 mg Pb/L. Results indicated that additional nitrogen, phosphates, and some carbon sources, including sodium acetate, citric acid and sodium carbonate, all protected the algae from Pb toxicity at 200 mg Pb/L. One hypothesis was that nutrients were able to reverse toxic effects. The second hypothesis was that nutrients directly interacted with Pb, in some way sequestering the metal so as to inhibit its metabolic interaction

with the organism (Rao and Reddy, 1985; Jampani, 1988). Rai and Raizada (1989) investigated the effects of Pb on nitrate and ammonium uptake and results indicated that Pb exposure can affect the uptake of some nutrients in *N. muscorum*. Thus, nutrients seem to be capable of reducing toxicity, though the mechanisms have not been well established.

Interactions with Other Pollutants

Predicting the response of organisms to mixtures of chemicals is a daunting task (Norwood et al., 2003). There are two major approaches to predict mixture toxicity including: (1) examining the combined mode of action of the individual mixture substances; and (2) determining whether an organism response to the mixture is additive, or some deviation from additive (synergistic or antagonistic). In addition, researchers may report mixture toxicity in terms of additive concentrations or additive effects, which can cause confusion in the interpretation of study results. For the studies presented in this section, the authors primarily report mixture toxicity in terms of additive concentrations (i.e., the sum of the concentrations of each individual chemical in the mixture will result in a level of effect similar to the simple sum of the effects observed if each chemical were applied separately).

When two or more metals compete for the same binding sites or interfere with transport through cell walls or membranes, the interaction is termed less than strictly additive or antagonistic. Antagonistic interactions can reduce metal bioavailability when metals are present in combination, and may lead to reduced potential for toxicity (Hassler et al., 2004). There are a number of elements (Ca^{2+}, Cd^{2+}, Mg^{2+}, Na^{+} and Cl^{-}) that act in an antagonistic fashion with Pb (Niyogi and Wood, 2004; Rogers and Wood, 2003, 2004; Ahern and Morris, 1998; Li et al., 2004). For example, Pb is a well-known antagonist to Ca^{2+} (Hassler et al., 2004; Niyogi and Wood, 2004). Calcium is an essential element, required for a number of physiological processes in most organisms.

Hassler et al. (2004) reported that in the presence of copper (Cu^{2+}) there was a significantly higher rate of internalization of Pb in the green algae *Chlorella kesserii*. It was suggested that Cu^{2+} may have affected organism physiology through the disruption of cell membrane integrity. This would allow increased cation (i.e., Pb^{2+}) permeability and therefore substantially increased internalization of Pb. Synergism is likely the result of increased bioavailability of one or more of the metal ions due to the presence of other metals (Hassler

et al., 2004).Synergistic interactions have also been observed with lead and other metals (Cd, Cu, Ni, and Zn) (Hagopian-Schlekat et al., 2001).

Norwood et al. (2003) reported, in a review and re-interpretation of published data on the interactions of metals in binary mixtures (n = 15 studies), that antagonistic (n = 6) and additive interactions (n = 6) were the most common for Pb. The two most commonly reported Pb-element interactions are between Pb and calcium and Pb and zinc. Both calcium and zinc are essential elements in organisms, and the interaction of Pb with these ions can lead to adverse effects both by increased Pb uptake and by a decrease in Ca and Zn required for normal metabolic functions.

7.2.4 Exposure/Response of Aquatic Species

Lead exposure may adversely affect organisms at different levels of organization, i.e., individual organisms, populations, communities, or ecosystems. Generally, however, there is insufficient information available for single materials in controlled studies to permit evaluation of specific impacts on higher levels of organization (beyond the individual organism). Potential effects at the population level or higher are, of necessity, extrapolated from individual level studies. Available population, community, or ecosystem level studies are typically conducted at sites that have been contaminated or adversely affected by multiple stressors (several chemicals alone or combined with physical or biological stressors). Therefore, the best documented links between Pb and effects on the environment are with effects on individual organisms.

Effects of Lead on Primary Producers

In the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986), several authors reported that some algal species (e.g., *Scenedesmus sp.*) were found to exhibit physiological changes when exposed to high Pb or organolead concentrations in situ. The observed changes included increasing numbers of vacuoles, deformations in cell organelles, and increased autolytic activity. Increased vacuolization was assumed to be a tolerance mechanism by which Pb was immobilized within cell vacuoles.

Several studies have been conducted since the 1986 Lead AQCD on the toxicity of Pb to primary producers (Rai and Raizada, 1989; Jampani, 1988; Adam and Abdel-Basset, 1990; Gaur et al., 1994; Gupta and Chandra, 1994). Effects to algal growth (*Chlorella vulgaris*, *Closterium*

acerosum, *Pediastrum simplex*, *Scenedesmus quadricauda*), ranging from minimal to complete inhibition, have been reported at Pb concentrations between 100 and 200,000 µg/L (Bilgrami and Kumar, 1997; Jampani, 1988). The toxicity of Pb to aquatic plant growth has been studied using *Spirodela polyrhiza*, *Azolla pinnata*, and *Lemna gibba* (Gaur et al., 1994; Gupta and Chandra, 1994; Miranda and Ilangovan, 1996). Test durations ranged from 4 to 25 days, and test concentrations ranged between 49.7 and 500,000 µg/L (Gaur et al., 1994; Miranda and Ilangovan, 1996). Research on aquatic plants has been focused on the effects of Pb on aquatic plant growth, chlorophyll and protein content.

Algae and other aquatic plants have a wide range in sensitivity to the effects of Pb in water. Both groups of primary producers experience EC_{50} values for growth inhibition between ~1,000 and >100,000 µg/L (Bilgrami and Kumar, 1997; Jampani, 1988; Gaur et al., 1994). The most sensitive primary producers reported in the literature for effects on growth were *Closterium acersoum* and *Azolla pinnata* (Bilgrami and Kumar, 1997; Gaur et al., 1994). The least sensitive primary producers reported in the literature for effects to growth were *Synechococcus aeruginosus* and *L. gibba* (Jampani, 1988; Miranda and Ilangovan, 1996). Exposure to Pb in combination with other metals generally inhibits growth less than exposure to Pb alone. Studies have shown that Pb adversely affects the metabolic processes of nitrate uptake, nitrogen fixation, ammonium uptake, and carbon fixation (Rai and Raizada, 1989). Lead in combination with nickel or chromium produced synergistic effects for nitrate uptake, nitrogenase activities, ammonium uptake, and carbon fixation (Rai and Raizada, 1989).

Effects of Lead on Consumers

The 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986) reported that hematological and neurological responses are the most commonly reported effects to aquatic vertebrates. These effects include red blood cell destruction and inhibition of the enzyme ALAD, required for hemoglobin synthesis. The lowest reported exposure concentration causing either hematological or neurological effects was 8 µg Pb/L (U.S. Environmental Protection Agency, 1986).

Recent literature on the toxicity of Pb to fish and aquatic invertebrates has been summarized by Eisler (2000). Exposure of invertebrates to Pb can lead to adverse effects on reproduction, growth, survival, and metabolism (Eisler, 2000). Water-borne Pb is highly toxic to

aquatic organisms, with toxicity varying, depending on the species and life stage tested, duration of exposure, the form of Pb tested, and water quality characteristics. Among the species tested, aquatic invertebrates (such as amphipods and water fleas) were the most sensitive to the effects of Pb with adverse effects being reported at concentrations as low as 0.45 µg/L (range: 0.45 to 8000 µg/L). Freshwater fish demonstrated adverse effects at concentrations ranging from 10 to >5400 µg/L, generally depending upon water quality parameters (e.g., pH, hardness, salinity). Amphibians tend to be relatively tolerant of Pb; however, they may exhibit decreased enzyme activity (e.g., ALAD reduction) and changes in behavior (e.g., hypoxia response behavior) (Eisler 2000). Lead tends to be more toxic in longer-term exposures, with chronic toxicity thresholds for reproduction in water fleas ranging as low as 30 µg/L (e.g., Kraak et al., 1994).

7.2.5 Effects of Lead on Natural Aquatic Ecosystems

The effects of Pb on natural aquatic ecosystems were examined for this report following the conceptual framework developed by the EPA Science Advisory Board (Young and Sanzone, 2002). The essential attributes used to describe ecological condition include landscape condition, biotic condition, chemical and physical characteristics, ecological processes, hydrology and geomorphology and natural disturbance regimes. For the biotic condition, the Science Advisory Board (SAB) framework identifies community extent, community composition, trophic structure, community dynamics, and physical structure as factors for assessing ecosystem health. The majority of the published literature pertaining to Pb and natural aquatic ecosystems focuses on the biotic condition and identifies effects on energy flow or nutrient cycling, community structure, community level effects, and predator-prey interactions. Other factors for assessing the biotic condition such as effects of Pb on species, populations, and organism conditions (e.g., physiological status) were discussed earlier in Sections 7.2.3 and 7.2.4 (see also Annex Sections AX7.2.3 and AX7.2.4).

Recent studies have attributed the presence of Pb to reduced primary productivity, respiration, and alterations of community structure. Specifically, Pb (6 to 80 mg/L) was found to reduce primary productivity and increase respiration in an algal community (Jayaraj et al., 1992). Laboratory microcosm studies have indicated reduced species abundance and diversity in protozoan communities exposed to 0.02 to 1 mg Pb/L (Fernandez-Leborans and Novillo, 1992, 1994; Fernandez-Leborans and Antonio-García, 1988). Numerous field studies have associated

the presence or bioaccumulation of Pb with reductions in species abundance, richness, or diversity, particularly in benthic macroinvertebrate communities (Deacon et al., 2001; Mize and Deacon, 2002; Mucha et al., 2003; Poulton et al., 1995; Rhea et al., 2004; Maret et al., 2003). However, in natural aquatic ecosystems, Pb is often found coexisting with other metals and other stressors. Thus, understanding the effects of Pb in natural systems is challenging given that observed effects may be due to cumulative toxicity from multiple stressors.

Exposure to Pb in laboratory studies and simulated ecosystems may alter competitive behaviors of species, predator-prey interactions, and contaminant avoidance behaviors. Alteration of these interactions may have negative effects on species abundance and community structure. For example, Pb concentrations ranging from 0.3-1.0 mg/L altered feeding behavior and affected predator avoidance in mummichogs (Weis and Weis 1998). Lead concentrations ranging from 0.5-1.0 mg/L altered feeding behavior in fathead minnows (Weber 1996), but did not elicit an avoidance response in American toads (Steele et al., 1991). The feeding behaviors of competitive species in some aquatic organisms (e.g., snails and tadpoles) are also influenced by the presence of Pb (Lefcort et al., 2000).

The effects of Pb have primarily been studied in instances of point source pollution rather than area-wide atmospheric deposition. Thus, the effects of atmospheric Pb on aquatic ecological condition remain to be defined. There is a paucity of data in the general literature that explores the effects of Pb in conjunction with all or several of the various components of ecological condition as defined by the EPA (Young and Sanzone, 2002). However, numerous studies are available associating the presence of Pb with effects on biotic conditions.

7.3 CRITICAL LOADS FOR LEAD IN TERRESTRIAL AND AQUATIC ECOSYSTEMS

This section defines critical loads, describes various concepts and methods that are related to the estimation of critical loads, and provides a review of the relevant literature on critical loads.

7.3.1 Definitions

Critical loads are defined in a variety of ways depending on the chemicals and endpoints of concern (Pačes, 1998; Skeffington, 1999; U.S. Environmental Protection Agency, 2004). For the purposes of this section, critical loads are defined as threshold deposition rates of air pollutants that current knowledge indicates will not cause long-term adverse effects to ecosystem structure and function. A critical load is related to an ecosystem's sensitivity to anthropogenic inputs of a specific chemical. If future inputs of a chemical exceed the critical load for an ecosystem, the chemical is expected to reach or persist at potentially toxic levels in the future. A critical load indicates a potential for future impacts only; a current exceedance of a critical load does not specify whether the current deposition rate of a chemical presents a hazard to the ecosystem. A current exceedance may or may not indicate a potential hazard; for the management application in which the critical load concept is applied, the calculation is for evaluating future impacts.

In order to determine a critical load, the lowest concentration in the receiving medium that poses a potential hazard to a defined ecosystem must first be determined. This concentration, known in the critical loads literature as the critical limit (De Vries et al., 2004), is equal to the effects-based criteria for the most sensitive endpoint in the ecosystem. The critical limit indicates the current potential for adverse effects to an ecosystem.

In contrast to a critical load, a stand-still load is the highest deposition rate of a chemical that will not result in future increases of its concentrations in the environmental media, regardless of the potential for adverse effects at those concentrations. Stand-still loads are also called "acceptable loads" or critical loads calculated using a "stand-still" approach (De Vries et al., 2004) and should not be confused with effects-based critical loads.

7.3.2 Historical Perspective

In the 1960s, scientists demonstrated that sulfur emissions on the European continent were contributing to the acidification of Scandinavian lakes. During the 1970s, evidence mounted that air pollutants could travel thousands of miles before deposition occurred, implying that international cooperation was necessary to control acidification. To this end, the European Community (EC) and 34 governments signed the *Convention on Long-range Transboundary of*

Air Pollution (CLRTAP) in 1979 under the auspices of the United Nations Economic Commission for Europe (United Nations Economic Commission for Europe (UNECE), 2004a; 2004b).

CLRTAP has since been extended to include eight protocols that regulate air pollutants such as sulfur, nitrogen oxides, heavy metals, persistent organic pollutants, volatile organic compounds, and ozone. In 1988, CLRTAP adopted the critical-load concept, making it basic to the future development of international agreements concerning limitation of the emissions of air pollutants. In 1991, The Coordination Center for Effects (CCE) issued a Technical Report entitled "Mapping Critical Loads for Europe" which presented the first maps of critical loads that were produced as part of the work conducted under the UNECE. Each individual country created maps detailing critical loads and levels of acidity within its boundaries. The maps were then used by CCE to create a Europe-wide map of critical loads (Hettelingh et al., 1991) that is used in combination with air emissions and deposition data to guide negotiations between nations and reduce the gap between critical loads and deposition (Skeffington, 1999). The first international agreement on pollution control based on critical loads was the second Sulfur Protocol, which was established in Oslo (United Nations Economic Commission for Europe (UNECE), 1994) within CLRTAP.

Since 1991, CCE has issued biennial technical status reports on critical loads and critical thresholds of acidification, eutrophication, sulfur, nitrogen, and nitrogen oxide (Coordination Center for Effects (CCE), 2005). Progress on data and methodologies is reviewed annually in CCE Mapping workshops. Recent CCE reports focus on scientific and technical support for the revision of protocols as well as time horizons for recovery from ecosystem damage.

Many of the signatory governments to CLRTAP have adopted the critical load concept for determining national emission control polices. Canada has also committed to a critical load approach for controlling acid deposition. In 1998, federal, provincial, and territorial Energy and Environment Ministers signed *The Canada-wide Acid Rain Strategy for Post-2000*. According to Environment Canada, the primary long-term goal of the *Strategy* is to achieve critical loads (or the threshold level) for acidic deposition across Canada (Environment Canada, 2003).

The Ministry of Environment in the Netherlands took the initiative to develop analogous methods for the calculation of critical loads for heavy metals, methods that would be valid in the context of CLPTRP (De Vries et al., 2004). Beginning in the mid-1990s, these methods were

developed through a series of manuals, international workshops, and expert meetings (De Vries et al., 2004). Participating nations completed a voluntary preliminary critical load mapping exercise for Pb and cadmium in Europe in 2002 (Hettelingh et al., 2002).

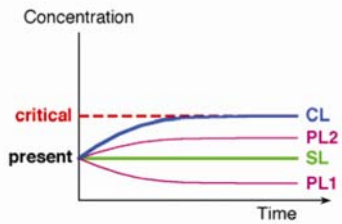
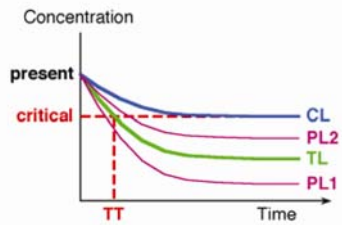
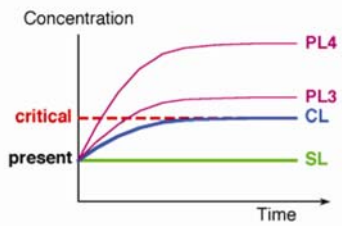
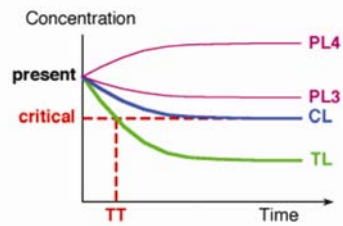
Germany has the National Focal Center (NFC) which coordinates and funds critical load applications for Germany through a private company (www.okekodata.com). That site presents preliminary data and applications of critical loads for several heavy metals in Sachsen and Nordrhein-Westphalen.

The 1986 Pb AQCD (U.S. Environmental Protection Agency, 1986) largely predates the development of the concept of critical loads and did not discuss this topic. The 2004 AQCD for Particulate Matter (U.S. Environmental Protection Agency, 2004) includes a brief discussion of the key elements of the critical loads framework as generally relevant to any air pollutant. To date, the critical loads framework has not been used for regulatory purposes in the United States for any chemical.

7.3.3 Application of Critical Loads to Terrestrial and Aquatic Ecosystems

A combinatorial application of critical limit and critical load allows one to assess current risk while simultaneously estimating future risk from exposure to a chemical (De Vries et al., 2004). Figure 7-1 shows that four combinations of critical load and limit exceedance or non-exceedance are possible for a given ecosystem (Figure 1 of De Vries et al. [2004]). For example, if a current risk is indicated by an exceedance of the critical limit for Pb due to historical Pb deposition, but current inputs of Pb to the ecosystem are below the critical load (upper right corner), the critical load model predicts that Pb concentrations will fall below the critical limit at some point in the future if Pb deposition is maintained at the present level. If current soil concentrations are below the critical limit (lower left corner), inputs greater than the critical load will not result in exceedance of the critical limit for some period of time, but continued exceedance of a critical load will eventually lead to an exceedance of the critical limit.

The time until a critical limit is exceeded (critical time) can also be predicted using the critical load model (Pačes, 1998). This requires knowledge of current concentrations, the critical load, and predicted deposition rates. Critical times may be useful for setting priorities between ecosystems with critical load exceedances or between different chemicals.

	No critical limit exceedance	Critical limit exceedance
No critical load exceedance	***No damage at present or foreseen:***  ➔ Keep the Present Load (more stringent than Critical Load)	***Present damage but recovery in progress:***  ➔ Keep the Present Load (more stringent than Critical Load) or ➔ Consider Target Load to reach the critical limit in a defined time period (more stringent than Critical Load)
Critical load exceedance	***Future damage foreseen:***  ➔ Consider Critical Load (emissions must decrease, even if concentrations in the ecosystem are allowed to increase further at critical load)	***Present damage, no recovery foreseen:***  ➔ Consider Critical Load (decrease of concentrations in the ecosystem down to critical limit in the long term) or ➔ Consider Target Load to reach the critical limit in a defined time period (more stringent than Critical Load)

CL - Critical load; PL - present load (2 cases); SL - Stand-still load; TL - Target load; TT - Target time

Figure 7-1. The predicted development of metal concentrations in ecosystems for four cases of exceedance or non-exceedance of critical limits and critical loads of heavy metals, respectively.

Source: Taken from DeVries et al. (2004).

7.3.4 Calculation of Critical Loads

This section summarizes the various methods used to calculate critical loads (De Vries et al., 2001, 2002, 2004; Groenenberg et al., 2002), with an emphasis on the most recent material.

7.3.4.1 Critical Limits

To determine the critical limit, effects-based criteria for the major ecological endpoints should be developed for the ecosystem of concern. Criteria may be developed for any receptor that is exposed to the chemical of concern deposited in the ecosystem. In terrestrial ecosystems, possible ecological endpoints include effects from direct contact of invertebrates or plants with soil and ingestion of plants by herbivores. Effects-based criteria for use in defining the critical limit should be derived from ecotoxicological data appropriate to the most sensitive endpoint (De Vries et al., 2004). Regardless of the selected endpoint, the critical limit should be defined as a concentration in the medium that receives the depositional load, typically soil in terrestrial ecosystems and surface water in aquatic ecosystems. To derive these values, uptake and/or food- chain modeling may be necessary.

Many critical load calculations rely on ecological effects criteria developed by government agencies in individual countries (Pačes, 1998; De Vries et al., 1998; Van Den Hout et al., 1999; Skjelkvåle et al., 2001). Criteria for Pb vary widely and can be the largest source of uncertainty in a critical load calculation (Van Den Hout et al., 1999). One reason for the wide range in estimates of effects criteria is that Pb speciation is often not taken into account. This can result in variation in estimates of concentration for total Pb that is associated with adverse effects, since the fraction of Pb available to cause a toxic effect depends on chemical factors such as the pH or organic matter content (Lofts et al., 2004). To develop effects-based criteria that are applicable to media with a pH or organic matter content different from the test medium, it is more appropriate to develop criteria based on the free concentration of Pb rather than the total concentration of Pb. For example, Figure 7-2 shows the relationship between the critical limit of Pb in soil as a function of organic matter content and pH.

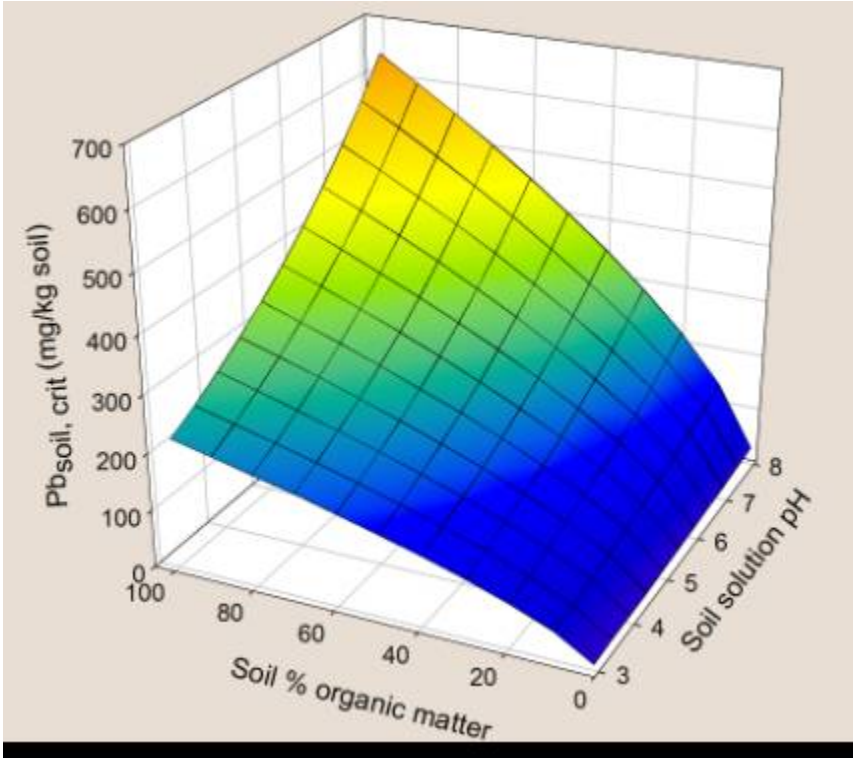


Figure 7-2. The relationship between the critical limit of Pb in soil as a function of organic matter and pH.

Source: Adapted from Lofts et al. (2004)
(http://www.york.ac.uk/depts/eeem/research/projects/criticalloads(stage3)/critlimitsstage3.htm)

7.3.4.2 Models

Critical loads for heavy metals are typically calculated using a steady state model that ignores internal metal cycling and keeps the calculations as simple as possible (De Vries et al., 2004). The critical load is equal to the atmospheric input flux, which equals the sum of the output fluxes from the system minus the other input fluxes (e.g., weathering) when the concentration of Pb is at the critical limit. The input flux of heavy metals via weathering is sometimes neglected, because quantitative estimates are highly uncertain, and weathering is generally thought to be a relatively minor process (De Vries et al., 2004; Scudlark et al., 2005).

More complex methods may be used to calculate critical loads. For example, dynamic models can be used to model the change of concentrations in soil or water over time (Pačes, 1998). These models are most valuable when the time to steady state is very long compared to

the time of interest. Using these models, the critical load is the deposition rate that leads to concentrations equal to the critical limit as the model approaches steady state. Fate and transport models that include explicit modeling of internal cycling and other refinements that may lead to improved accuracy of the models can be used in place of simple mass-balance models (Doyle et al., 2003).

Terrestrial Model

If internal cycling and weathering of Pb is neglected and atmospheric deposition is the only important source of Pb to the system, the critical load in a terrestrial ecosystem is equal to the sum of the most important fluxes out of the system, leaching, and uptake by harvested plants:

$$CL(Pb) = Pb_u + Pb_{le(crit)} \tag{7-1}$$

where:

$CL(Pb)$	=	critical load of Pb (mass per area-year)
Pb_u	=	metal net uptake in harvestable parts of plants at the critical limit (mass per area-year)
$Pb_{le(crit)}$	=	leaching flux of Pb (dissolved and particulate) from the soil layer at the critical limit (mass per area-year)

When applying a mass balance model, it is important to define the boundaries of the compartment such that all significant fluxes in and out of the compartment can be accounted for.

Uptake of Pb by harvested vegetation may be an important flux out of agricultural soil or forested soil that is actively logged. In ecosystems that are not harvested, the steady state model assumes that uptake by plants is balanced by deposition of Pb from decaying vegetation.

The flux out of the system due to uptake in harvested plants (Pb_u) is calculated as follows:

$$Pb_u = f_{Pb,u,z} * Y_{ha} * [Pb]_{ha} \tag{7-2}$$

where:

$f_{Pb,u,z}$	=	fraction of net Pb uptake from soil within the considered layer (dimensionless)
Y_{ha}	=	annual yield of harvestable biomass (mass per area-year)
$[Pb]_{ha}$	=	metal concentration of harvestable parts of plants (Pb per unit mass)

The net fraction of metal uptake from soil within the considered layer corrects for Pb measured in harvested vegetation that is taken up via direct deposition onto the plant or from soil outside of the considered soil layer.

The yield of harvestable biomass should only include the parts of plants that are removed from the system. Tree leaves, stalks remaining after harvest of agricultural land, roots, and other parts that remain in the considered terrestrial ecosystem should not be included in the yield.

De Vries et al. (2004) recommends that data for metal content in harvestable biomass should be taken from unpolluted areas. If the selected endpoint for the critical limit is related to the concentration in harvested plants rather than a concentration in soil, that critical concentration should be used in place of actual metal content in harvestable biomass.

The critical leaching flux from the topsoil can be calculated as follows:

$$Pb_{cl(crit)} = Q_{le} * [Pb]_{tot,sdw(crit)} \quad (7\text{-}3)$$

where:

Q_{le}	=	flux of drainage water leaching from the considered soil layer (volume/year)
$[Pb]_{tot,sdw(crit)}$	=	critical total concentration of Pb in soil drainage water (mass per volume)

The total concentration of Pb in soil drainage water is the sum of all species of dissolved and particulate Pb that leach out of the system in drainage water. De Vries et al. (2004) suggests that Pb that is sorbed to suspended particulate matter should be neglected so that total Pb is equal to dissolved Pb, as concentrations of suspended solids are difficult to estimate. Dissolved Pb may exist as free ions, organic complexes, or inorganic complexes.

The drainage water flux leaching from the topsoil (Q_{le}) can be calculated as follows:

$$Q_{le} = P - E_i - E_s - f_{Et,z} * E_t \quad (7\text{-}4)$$

where:

P	=	Precipitation (volume per area-time)
E_i	=	Interception evaporation (volume per area-time)
E_s	=	Soil evaporation within the topsoil (volume per area-time)
$f_{Et,z}$	=	Plant transpiration (volume per area-time)
E_t	=	Fraction of water uptake within the topsoil by roots (unitless)

De Vries et al. (2004) recommends default values for some of these parameters and provides an alternative calculation method for sites with detailed hydrologic data as part of the guidance document.

Aquatic Model

If internal cycling and weathering of Pb is neglected and atmospheric deposition is the only important source of Pb to the system, the critical load in an aquatic ecosystem is equal to the sum of the most important fluxes out of the system, uptake by harvested plants in the catchment, sedimentation, and lateral outflow from the catchment:

$$CL(Pb) = Pb_u + Pb_{sed(crit)} * A_l / A_c + Pb_{loc,crit} \quad (7\text{-}5)$$

where:

$CL(Pb)$	=	critical load of Pb (mass per area-year)
Pb_u	=	removal of Pb by harvesting of vegetation in the catchment (mass per area-time)
$Pb_{sed(crit)}$	=	removal of Pb by sedimentation at the critical load (mass per area-time)
$Pb_{loc,crit}$	=	lateral Pb outflow from the catchment at the critical load (mass per area-time)
A_l	=	lake area
A_c	=	catchment area

It is important to carefully define the boundaries of the aquatic system, so that all inflows and outflows may be fully accounted for. Current guidance recommends including the entire watershed within the system, rather than confining the system to a single lake or stream (De Vries et al., 2004). In stream water, removal of Pb due to sedimentation does not need to be considered, simplifying the equation to the following:

$$CL(Pb) = Pb_u + Pb_{loc,crit} \quad (7\text{-}6)$$

De Vries et al. (2004) recommends that critical loads should be calculated for stream waters only, due to a high level of uncertainty in the rate of removal via sedimentation or other removal mechanisms within a lake. Critical loads for streams are protective of nearby lakes,

because the critical loads calculated using this methodology will be lower for streams than for lakes.

Calculation of removal of Pb by harvesting of vegetation in the catchment is similar to that in terrestrial ecosystems, with $f_{Pb,u}$ equal to 1, since the entire catchment is now included.

The critical lateral Pb outflow from the catchment is the product of the lateral outflow flux of water and the total concentration of Pb in the outflow water at the critical limit. The outflow flux of water is calculated from the outflow divided by the catchment area.

7.3.5 Critical Loads in Terrestrial Ecosystems

Critical loads of Pb have been calculated using simple mass balance, dynamic, and probabilistic models for forested and agricultural land in Europe and Canada in a handful of preliminary studies. The methods and model assumptions used to calculate critical loads vary widely between these studies and little attempt has been made to validate the models that were used, so it is not known how much various simplifying assumptions affect the results.

Pačes (1998) used data from a small agricultural catchment in the Czech Republic that is typical of agricultural land in that country to calculate critical loads for Pb and other heavy metals. The critical loads were calculated using a simple dynamic box model. The fluxes into the system included atmospheric deposition, agricultural inputs, and weathering of bedrock and the fluxes out of the system included biological uptake and runoff. The model assumed that inputs of metals to the system are independent of their concentrations in soil but that outputs are proportional to the concentration of biologically active metal. The author defined biologically active metal as the concentration of metal in soil that can be extracted in a 2 M nitric acid solution. This method was used to set a Czech state norm designed to be protective for soil systems that is used as the critical limit in this study. Using the model, Pačes determined that the critical limit was not presently exceeded, but that the critical load is exceeded. However, the critical time was almost 1,000 years. Therefore, the model predicts that Pb will continue to accumulate in Czech agricultural soil and will eventually pose a potential risk if current inputs continue. The author identified the simplifying assumptions used to calculate fluxes out of the system as the major source of uncertainty.

Van den Hout et al. (1999) calculated critical loads for Pb and other pollutants in the organic and mineral soil layers of forested ecosystems. Atmospheric deposition was assumed to

be the only inflow, and outflows from soil were assumed to occur due to biological uptake and leaching. Net heavy metal uptake by the forest was set equal to the rate of water uptake by vegetation multiplied by the water concentration and a "preference factor" that indicates the preference of the vegetation for the metal relative to water. Water flux was estimated from precipitation, soil evaporation, and transpiration data. An equilibrium speciation model that takes inorganic and organic ligands into account was used to estimate dissolved concentrations of Pb in leachate. Results were strongly dependant on the critical limits that were chosen. Using the most stringent levels, critical loads were exceeded over much of Europe. The time to steady state was estimated to be hundreds of years. Speciation of Pb was identified as an important source of uncertainty.

Reinds et al. (2002) used the guidance prepared by De Vries et al. (2002b) to calculate critical loads in the mineral topsoil of forested and agricultural ecosystems across 80,000 acres of the European continent. The median critical load for Pb in Europe was 25 g ha^{-1} $year^{-1}$ using this methodology. The drainage water flux leaching from the topsoil was the dominant term in the model, so critical loads followed the spatial pattern of net runoff (excess precipitation) across Europe.

Probst et al. (2003) calculated critical loads for Pb for forested sites in France. Weathering rates were determined using a model for representative French soil samples. The biomass uptake of Pb was derived using National Forestry Inventory data for the average annual biomass growth and data for the Pb content in biomass. An uptake factor scaled down to the considered depth was applied. Leaching of Pb was calculated using runoff data and dissolved Pb concentrations in soil solution. Critical loads at the French site varied over a wide range (4.9 to 133 g ha- $year^{-1}$). Critical loads were controlled mainly by net runoff. Weathering rates were small compared to leaching and biomass uptake rates.

Doyle et al. (2003) used a probabilistic assessment to calculate critical loads in terrestrial and aquatic (see following section) ecosystems on the Canadian Shield. The terrestrial model used an analytical solution to the convection/dispersion equation. The model only considered soluble metal in the flux to soil and assumed that the insoluble fraction was not available. Metals were assumed to be sorbed onto immobile soil solids according to an equilibrium distribution (Kd) relationship. The input parameters were selected to represent boreal forest and Canadian Shield conditions. Best estimate inputs were used for deterministic evaluation and distributions

of values were used in a probabilistic assessment. The model inputs included net water flux, effective water velocity, moisture content of soil, pH, dispersion coefficient, and Kd. The 25th percentile critical loads (47 mg/m^3 per year for Pb) were compared to current deposition rates to evaluate risk.

In spite of the variation in methods and model assumptions used to calculate critical loads for Pb in the studies discussed above, some general conclusions may be drawn. The critical limit is the most important value for determining the value of the critical load. Wide variations in available effects levels makes this parameter one of the most important sources of uncertainty when calculating critical loads in terrestrial ecosystems. Spatial variations in critical loads for Pb are largely controlled by net runoff. Weathering and uptake by harvestable vegetation were less important. The time to reach steady state is several hundred years in the two studies that used dynamic models to determine critical loads.

7.3.6 Critical Loads in Aquatic Ecosystems

Doyle et al. (2003) modeled critical loads in surface water bodies assuming complete mixing with dilution water entering from the terrestrial catchment area. Loss of metal was also assumed to occur through downstream flushing and burial in sediment. Transfer of metal to sediment was modeled as a first-order process dependant on the dissolved concentration and pH. The inputs to the model included the following: water body area, terrestrial catchment area, water body depth, sediment accumulation rate, thickness of biologically active sediment, net precipitation, and water pH. The fist-order rate constant for transfer to sediment was correlated with pH. The model reached steady state within a few years. Transfer of Pb from the terrestrial catchment to the water body was neglected, because the time to steady state could be on the order of 10,000 years if the model included this source of Pb. However, the authors cited a separate calculation that indicated that neglect of transfer of Pb from the catchment may lead to a 5-fold underestimation of Pb concentrations in the surface water.

These results indicate that Pb run-off from soil is more important than direct atmospheric deposition to the surface water bodies considered in this study. Due to the long times required to achieve steady state, the critical load methodology may not be appropriate for Pb in aquatic systems.

7.3.7 Limitations and Uncertainties

The largest sources of uncertainty identified in studies of critical loads for Pb include the following:

- Steady-state assumption
- Derivation of the critical limit
- Lead speciation
- Soil runoff as an input to aquatic ecosystems

The critical load is calculated for steady state conditions, but the time for Pb to reach steady-state concentrations can be as long as several centuries. Thus, dynamic models are often used to predict Pb concentrations over shorter time frames. Dynamic modeling requires additional knowledge about current concentrations in the considered ecosystem. For regulatory purposes, use of dynamic modeling requires that a target time be set in order to calculate a critical load.

Criteria for the protection of soil and for the protection of aquatic organisms vary over a wide range from country to country. Use of the critical loads method for international negotiations will require implementation of a consistent calculation methodology that takes into account the effect of Pb speciation on toxicity over a range of soil types and chemical conditions.

Speciation strongly influences the toxicity of Pb in soil and water and partitioning between dissolved and solid phases determines the concentration of Pb in soil drainage water, but it has not been taken into account in most of the critical load calculations for Pb performed to date. Recent guidance for heavy metals has begun to emphasize the importance of speciation to critical load calculations and suggest methods to calculate speciation (De Vries et al., 2004). To this end, Lofts et al. (2004) developed critical limit functions for several metals, including Pb, that take into account the effects of pH, organic matter, and the protective effects of cations on speciation.

Runoff of Pb from soil may be the major source of Pb into aquatic systems. However, little attempt has been made to include this source into critical load calculations for aquatic systems due to the complexity of including this source in the critical load models.

7.3.8 Conclusions

Preliminary efforts to calculate critical loads for Pb in terrestrial and aquatic ecosystems have so far relied on a variety of calculation methods and model assumptions. Efforts are ongoing to refine and standardize methods for the calculation of critical loads for heavy metals which are valid in the context of CLPTRP. At this time, the methods and models commonly used for the calculation of critical loads have not been validated for Pb. Many of the methods neglect the speciation of Pb when estimating critical limits, the uptake of Pb into plants, and the outflux of Pb in drainage water, limiting the utility of current models.

Future efforts should focus on fully incorporating the role of Pb speciation into critical load models, and validating the assumptions used by the models.

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8. INTEGRATIVE SYNTHESIS: MULTIMEDIA LEAD EXPOSURE, HUMAN HEALTH EFFECTS, AND ECOSYSTEM EFFECTS

8.1 INTRODUCTION

This integrative synthesis is structured to provide a coherent framework to support the assessment of multimedia exposures, human health risks, and ecological effects associated with ambient airborne lead (Pb) in the United States. The main goal of the chapter is to integrate newly available scientific information with key findings and conclusions from the 1986 Air Quality Criteria Document (Lead AQCD) and its associated Addendum (U.S. Environmental Protection Agency, 1986a,b), and their 1990 Supplement (U.S. Environmental Protection Agency, 1990), to address issues central to the EPA's assessment of evidence needed to support the current ongoing periodic review of the Pb NAAQS. The integrated assessment of key findings and conclusions provided here and elsewhere in this document provides key inputs to further analyses of such findings and their policy implications as delineated in a Lead Staff Paper prepared by EPA's Office of Air Quality Planning and Standards (OAQPS). The analyses provided in that Staff Paper aim to "bridge the gap" between scientific assessments in this criteria document and judgments required of the EPA administrator in evaluating whether to retain or, possibly, to revise the current primary and/or secondary Pb NAAQS.

8.1.1 Historical Background

In 1971, U.S. EPA promulgated national ambient air quality standards for several major "criteria" pollutants (see Federal Register, 1971), but did not include Pb among them at that time. Later, on October 5, 1978, the EPA promulgated primary and secondary Pb NAAQS under Section 109 of the CAA (43 FR 46258), as announced in the Federal Register (1979). Identical primary and secondary Pb standards were then established as: 1.5 $\mu g/m^3$ as a calendar quarterly average (maximum arithmetic mean averaged over 90 days). Those standards were based on scientific assessments in EPA's original *Air Quality Criteria for Lead* (U.S. Environmental Protection Agency, 1977) or "1977 Lead AQCD."

In 1986, the EPA published a revised Lead AQCD (U.S. Environmental Protection Agency, 1986a), which assessed newly available scientific information on health and welfare effects associated with exposure to various concentrations of Pb in ambient air, based on literature published through 1985. That 1986 document mainly assessed the health and welfare effects of Pb, but other scientific data were also discussed in order to provide a better understanding of the pollutant in the environment. Thus, the 1986 Lead AQCD included chapters that discussed the atmospheric chemistry and physics of the pollutant; analytical approaches; environmental concentrations; human exposure and dosimetry; physiological, toxicological, clinical, and epidemiological aspects of Pb health effects; and Pb effects on ecosystems. An Addendum to the 1986 Lead AQCD was also published along with it (U.S. Environmental Protection Agency, 1986b). Then, a Supplement to the 1986 Lead AQCD/Addendum was published by EPA in 1990 (U.S. Environmental Protection Agency, 1990a). That 1990 Supplement evaluated still newer information emerging in the published literature concerning (a) Pb effects on blood pressure and other cardiovascular endpoints and (b) the effects of Pb exposure during pregnancy or during the early postnatal period on birth outcomes and/or on the neonatal physical and neuropsychological development of infants and children.

The 1986 Lead AQCD/Addendum and the 1990 Supplement provided scientific inputs to support decision-making regarding CAA-mandated periodic review and, as appropriate, revision of the Pb NAAQS; and they were drawn upon in preparation of an associated OAQPS Lead Staff Paper (U.S. Environmental Protection Agency, 1990b). Based on scientific assessments in the 1986 Lead AQCD/Addendum and the 1990 Supplement, as well as associated exposure/risk analyses, the 1990 Staff Paper recommended that the EPA Administrator consider options for revision of the Pb NAAQS to a level in the range of 0.5 to 0.75 $\mu g/m^3$ (30-day arithmetic mean). After considering those evaluations, EPA chose not to propose revision of the Pb NAAQS. At the time, as part of implementing a broad 1991 U.S. EPA Strategy for Reducing Lead Exposures (U.S. Environmental Protection Agency, 1991), the Agency focused primarily on regulatory and remedial clean-up efforts aimed at reducing Pb exposures from a variety of non-air sources judged to pose more extensive public health risks to U.S. populations, as well as on other actions to reduce Pb emissions to air. By 1990, average ambient air Pb levels had dropped to 0.15 to 0.25 $\mu g/m^3$ across U.S. urban areas due to the phasedown of Pb in gasoline.

8.1.2 Chapter Organization

The ensuing chapter sections collectively address the following topics: (1) ambient airborne lead compounds, sources, emissions, and air quality; (2) ambient Pb exposures pathways and dosimetric considerations; (3) epidemiologic and toxicologic evidence for associations between Pb exposure of human populations and various health effects, demonstrating a broad array of pathophysiologic responses of humans and animals to acute and chronic Pb exposures; (4) characterization of applicable dose-response relationships for various types of Pb-exposure effects; (5) persistence of key Pb exposure effects; (6) factors that enhance or lessen susceptibility or vulnerability to Pb health effects; (7) identification of susceptible and vulnerable human population groups likely at increased risk for Pb-related health effects; (8) potential public health implications of low-level Pb exposures; and (9) delineation of ecological effects of Pb.

8.2 OVERVIEW OF MULTIMEDIA LEAD, SOURCES, EMISSIONS, AND CONCENTRATIONS IN THE UNITED STATES

Lead has been observed in measurable quantities in nearly every environmental medium all over the world. Human exposure to Pb occurs through several routes, as shown in Figure 8-1, which provides a simplified diagram of various routes of exposure through different environmental media, with a main focus on the ambient air. The multimedia aspects of Pb exposure can be seen in that Pb emissions to the air contribute to Pb concentrations in water, soil, and dusts; Pb in soil and dust also can make important contributions to Pb concentrations in ambient air. The relative contributions of Pb from different media and different sources on human exposure depend on factors such as the proximity of major sources to the residence and workplace of the individual, the condition of the residence (especially the presence and condition of lead-based paint) and whether the residence is in an urban, suburban, or rural location. This section briefly summarizes available evidence concerning multimedia Pb sources and exposure pathways, with main emphasis on pathways involving airborne Pb components.

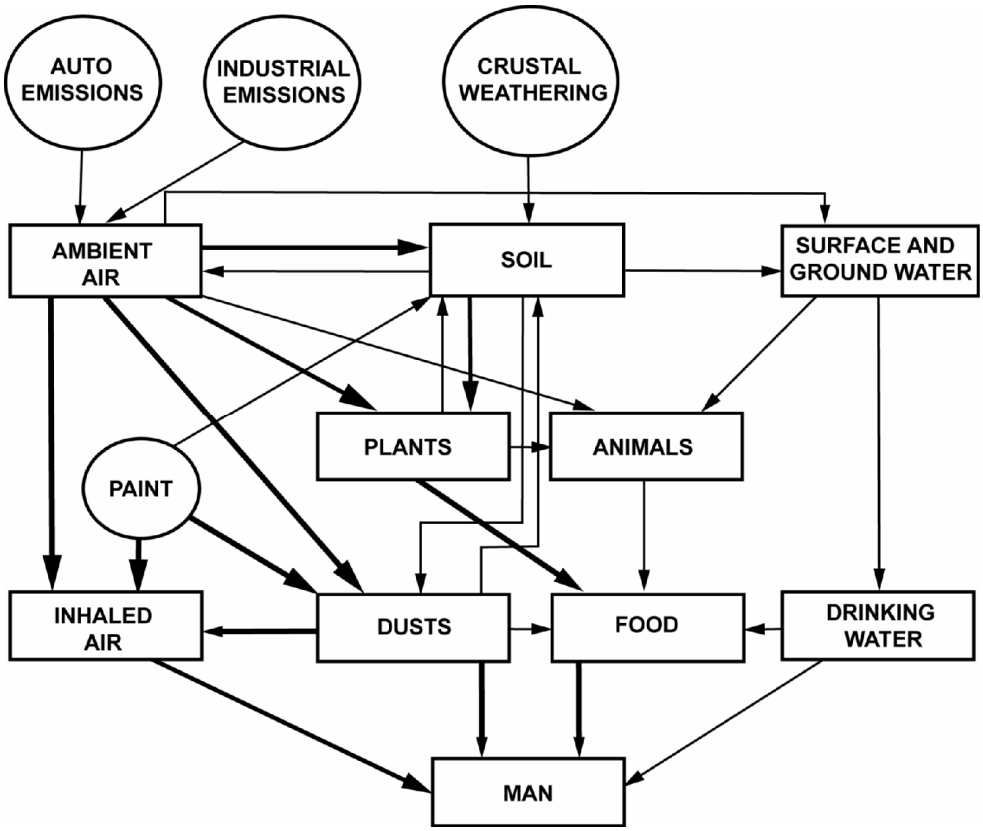


Figure 8-1. Principal pathways of lead from the environment to human consumption. Heavy arrows are those pathways discussed in greatest detail in this chapter.

8.2.1 Sources of Lead Emissions into Ambient Air

In ambient air, Pb occurs mainly as a component of organometallic compounds and various salts or other compounds (as summarized in Chapter 2, Section 2.1) rather than as elemental Pb because, at ambient atmospheric temperatures, elemental Pb deposits to surfaces or forms a component of atmospheric aerosol. Those salts and covalently bound Pb compounds that are of significance in the environment include: sulfates ($PbSO_4$); chlorides ($PbCl_2$); carbonates ($PbCO_3$, $Pb(HCO_3)_2$); hydroxides ($Pb(OH)_2$); nitrates ($Pb(NO_3)_2$); phosphates ($PbPO_4$, $Pb(HPO_4)_2$); oxides (PbO, Pb_3O_4), silicates, and PbS. With the exception of the covalently-bound sulfide and oxide, these compounds are derived from acids (or the related anions) that are common in the environment, such as sulfuric acid (H_2SO_4), nitric acid (HNO_3), carbonic acid (H_2CO_3, an acid that forms when CO_2 dissolves in water), and phosphoric acid

(H_3PO_4). Lead salts, once formed, tend to be only slightly soluble in neutral solutions, but are quite soluble in the presence of acid. Another form of Pb-containing compounds is the tetravalent Pb (IV) organometallic compounds, such as the well-known fuel additives, tetramethyllead (TML) and tetraethyllead (TEL).

Natural sources of Pb emissions to the air include volcanoes, sea-salt spray, biogenic sources, forest fires, and wind-blown soil. There is significant variability in Pb emissions from these sources, but it has been estimated that they contribute 10 to 20 thousand tons per year in annual emissions of Pb, worldwide (see Chapter 2, Section 2.2.1). In addition to the typically relatively limited inputs of natural sources to ambient air, Pb emitted into the air from a wide variety of anthropogenic sources can contribute to human exposure via a number of often interlinking multimedia exposure pathways, as illustrated in Figure 8-1 and discussed below.

Historically, mobile sources constituted a major source of Pb emissions into the ambient air, due to the use of leaded gasoline (Section 2.2.4). Although its phase down began in 1974, some Pb was still added to gasoline in the United States as an anti-knock additive at the time of 1986 Lead AQCD/Addendum. Accordingly, airborne Pb concentrations nationwide have fallen dramatically over the past 20 years; and this represents one of the most important public and environmental health successes in history. Remaining mobile source-related emissions of Pb include brake wear, resuspended road dust, and emissions from vehicles that continue to use leaded gasoline (e.g., some types of race cars and aircraft).

The dramatic decreases in Pb emissions to U.S. ambient air during recent decades, including the notable decreases in Pb emissions from mobile sources, are shown in Figure 8-2. Nationwide, ambient air Pb emissions fell 98% between 1970 and 2002 (U.S. Environmental Protection Agency, 2003), primarily due to elimination of alkyl lead additives to automotive gasoline. The decreasing contributions of mobile sources to ambient airborne Pb have been documented by National Emissions Inventory data for Pb emissions from various sources for the United States in 1990 and 2002 (see Table 2-8). In 1990, mobile sources still constituted the largest single source of U.S. Pb emissions, even though substantial reductions in airborne Pb had already occurred due to the phasedown of Pb in gasoline. However, the emissions inventory data from 2002 show that, while mobile sources continue to make some contributions to Pb emissions, industrial sources now play a much more significant proportional role (as can be seen in Table 2-8).

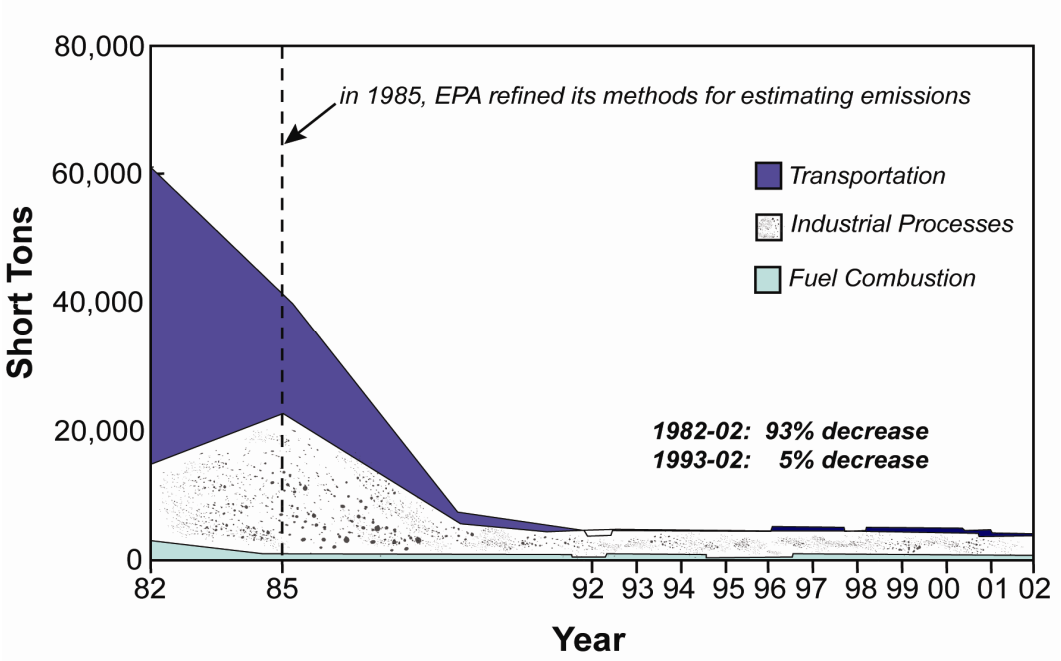


Figure 8-2. Trends in U.S. air lead emissions during the 1982 to 2002 period.

Source: U.S. Environmental Protection Agency (2003).

As discussed in Section 2.2.2, the largest Pb emitters into the ambient air are now in the manufacturing sector, which includes combustion sources (such as industrial or utility boilers and municipal or hazardous waste incinerators), iron and steel foundries, primary and secondary smelters, and other, mainly, stationary sources of Pb emissions to the air. Other stationary sources of airborne Pb emissions include smelters for other metals, such as copper or nickel, Pb-acid battery manufacturing, cement manufacturing and mining or processing of Pb.

One observation that can be drawn from the data on trends in Pb emissions is that currently the occurrence of airborne Pb concentrations in the United States is influenced heavily by localized industrial or other stationary sources of Pb, in contrast to the situation a few decades ago when elevated U.S. ambient air Pb concentrations were widespread mainly as a result of leaded fuel use.

8.2.2 Transport and Secondary Dispersal of Atmospheric Lead

Lead can be transported in the atmosphere and undergo secondary dispersal via the deposition and resuspension of particles containing Pb, as discussed in Section 2.3.2 of Chapter 2. As discussed in Section 2.3.1, numerous studies have analyzed Pb concentrations in media such as soil, sediments, ocean water, peat bogs, plants, snowpacks, or ice cores to evaluate the historical record of deposition of Pb. Sediments can provide records dating back several million years, peat bogs can reach back to the late glacial period (~15000 years ago), corals and trees can record up to several hundred years, and lichens and mosses can provide recent deposition data (Weiss et al., 1999). Many studies have shown a pattern of sediment Pb concentrations increasing to reach peak concentrations in layers representing deposition during the 1970's followed by marked declines in more recent years. For example, Figure 8-3 presents data on Pb concentrations in sediment samples from 12 lakes in the Great Lakes area (Yohn et al., 2004).

Deposition of Airborne Lead

Dry deposition is the process by which pollutants are removed from the atmosphere in the absence of precipitation. The size of depositing particles is arguably the most important factor affecting dry deposition rates. For very small particles, Brownian motion is the dominant mechanism that transports particles through the viscous sublayer that borders surfaces. For large particles, sedimentation is the most important process governing particle deposition. For intermediate particles, impaction and interception largely determine deposition rates. The highest extent of uncertainty applies to deposition velocities for the intermediate sized particles. As an example, in one study, although most of the airborne Pb mass was associated with submicron particles, only about 0.5% of the Pb particle mass undergoing dry deposition in Chicago was <2.5 μm in diameter. Also, more than 90% of Pb particle mass that undergoes dry deposition is in an insoluble chemical form. Overall, dry deposition velocities for Pb are in the range of 0.05 to 1.3 cm/s and dry deposition flux rates have been estimated to be in the range of ~1-2 mg/m^2-year.

Wet deposition is the process by which airborne pollutants are scavenged by precipitation and removed from the atmosphere. The size of particles can also influence wet deposition rates. Large particles are scavenged more efficiently. Lead, which is found in particles primarily in

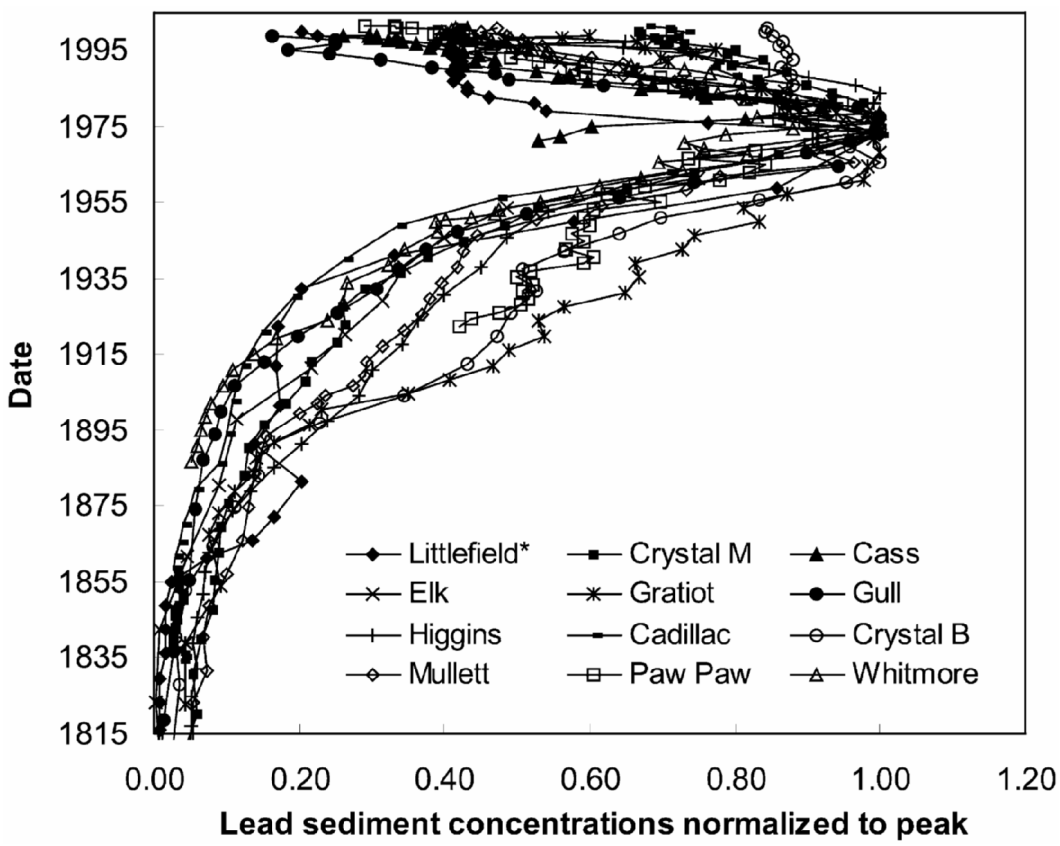


Figure 8-3. Lead concentrations in sediment samples in 12 Michigan lakes. The concentrations are normalized by the peak Pb concentration in each lake; peak Pb concentrations ranged from approximately 50 to 300 mg/kg.

Source: Yohn et al. (2004).

the submicron size range, does not undergo wet deposition as easily as many of the crustal elements. Wet deposition flux has been estimated to range from about 300-1000 $\mu g/m^2$-year in U.S. locations, as discussed in Section 2.3.2.

Resuspension of Lead in Soil

The resuspension of soil-bound Pb particles and contaminated road dust is a significant source of airborne Pb. The main sources of resuspension are typically wind and vehicular traffic, although resuspension through other mechanical processes, e.g., construction, pedestrian traffic,

agricultural operations, and even raindrop impaction, is possible. In general, mechanical stresses are more effective than the wind in resuspending particles.

Understanding the physics of resuspension from natural winds requires analyzing the wind stresses on individual particles, including frictional drag, form drag, gravitation, and the Bernoulli effect. Although this analysis can be accurate on a small scale, predicting resuspension on a large scale generally focuses on empirical data for continual soil movement due to three processes: saltation, surface creep, and suspension. Saltation is the process by which particles in the 100 to 500 μm size range bounce or jump close to the surface. The low angle at which these particles strike the surface transfers momentum to smaller particles, allowing them to be suspended into the atmosphere. Depending on soil conditions, saltation can be responsible for moving 50 to 75% of surface particles. Surface creep is the rolling or sliding motion of particles induced by wind stress or momentum exchanged from other moving particles. This generally applies to large particles 500 to 1000 μm in diameter and moves 5 to 25% of soil by weight. Suspension is the process that actually ejects particles into the air. This affects particles $\leq$100 μm in diameter and moves 3 to 40% of soil by weight. Resuspension may occur as a series of events. Short episodes of high windspeeds, dry conditions, and other factors conducive to resuspension may dominate annual averages of upward flux.

Soil-Pb concentrations vary significantly throughout urban areas, depending on proximity to roadways and stationary sources and on wind speed and direction, as noted in Section 3.2.1. Some of the highest soil-Pb concentrations are observed near major roadways. For example, surface soil-Pb concentrations measured near a major freeway in Cincinnati, OH, were between 59 ppm and 1980 ppm, levels well above background. These concentrations dropped off dramatically with soil depth. An estimated 40% of Pb from automobile exhaust was retained in the nearby soil. Lead-contaminated soils and dusts can be significant sources of Pb exposure for human populations.

Lead in soil is also highly elevated near stationary sources of Pb emissions. In particular, areas around smelters and battery disposal sites can have very high levels of soil Pb (Section 3.2.2). Concentrations of soil Pb are highly elevated near mines as well. Lead and zinc mines, in particular, typically have large deposits of Pb in nearby soil, but mines used for extracting other metals can also have Pb-contaminated soil. Blood-Pb levels are typically elevated in people living near Pb mines.

The resuspension of soil particles historically contaminated by past deposition of airborne Pb emitted from smelters and other stationary sources, as well as resulting from past combustion of leaded gasoline, represents a continuing source of current air Pb.

8.2.3 Ambient Air Lead Concentrations

There are four ambient monitoring networks that measure Pb concentrations in the United States, as discussed in Section 3.2.1 of Chapter 3. Determination of compliance with the current Pb NAAQS is based on measurements taken at Federal Reference Method (FRM) monitors, which measure Pb in total suspended particulate matter (TSP), i.e., particles up to about 30 µm in diameter. In 2005, there were about 250 FRM sampling sites in operation across the United States.

Data on airborne Pb concentrations are also available from two other U.S. networks that measure Pb in fine particulate matter (≤2.5 µm in diameter). There are ~200 sites, primarily in U.S. urban locations, in the $PM_{2.5}$ speciation network; and there are over 100 sites in the Interagency Monitoring of Protected Visual Environments (IMPROVE) network that are located in U.S. national parks or wilderness areas. In addition, Pb concentrations are measured in PM_{10} samples collected at the National Air Toxics Trends Stations (NATTS) network of 24 U.S. sites.

As was seen for emissions of Pb, ambient air Pb concentrations have also markedly declined over the past several decades. Between 1983 and 2002, ambient air Pb concentrations measured at FRM monitors decreased by ~94%, as shown in Figure 8-4. Data from the FRM monitors and from the $PM_{2.5}$ speciation, IMPROVE and NATTS networks all show a consistent pattern of ambient air Pb concentrations, i.e., a long period of measured ambient levels substantially lower than the current Pb NAAQS, except in a few local areas. For example, Pb concentrations measured at the FRM monitors in 2000 to 2004 are quite low, on average, with the mean level ranging from 0.03 to 0.05 $\mu g/m^3$ (excluding point source-related monitors) and 0.10 to 0.22 (including point source-related monitors). However, when data from point source-oriented monitors are included, one to five U.S. locations (from among ~200 sites) had measured calendar quarterly maximum Pb levels that exceeded the NAAQS level (1.5 $\mu g/m^3$, quarterly max average) in any given year during 2000 to 2004. As for data from PM_{10} monitors in the NATTS network, the highest quarterly max Pb concentration observed was 0.039 $\mu g/m^3$ during

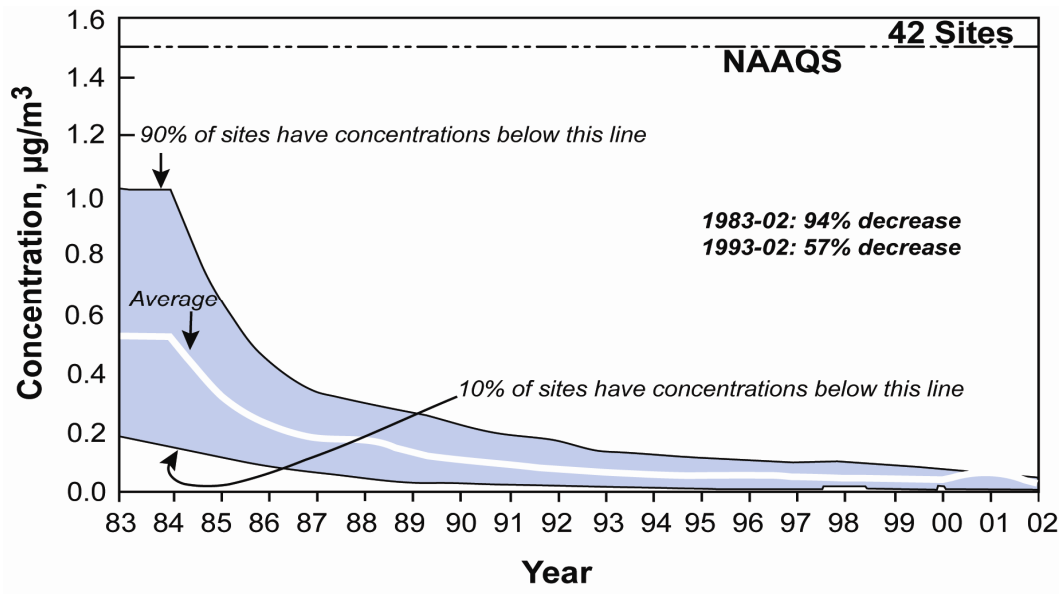


Figure 8-4. Airborne Pb concentrations measured at FRM sites, averaged across the United States for the years 1983 through 2002. The data are plotted in terms of maximum arithmetic mean averaged over a calendar quarter and are shown in relation to the Pb NAAQS of 1.5 µg/m^3

Source: U.S. Environmental Protection Agency (2003).

2002 to 2005. Using data from the $PM_{2.5}$ speciation network for 2002 to 2005, the highest quarterly max Pb concentration reported was 0.168 µg/m^3.

Descriptive statistics for Pb concentrations determined from several particle size fractions are presented in Table 8-1. Focusing on the Pb concentrations reported from TSP, PM_{10} and $PM_{2.5}$ samples in urban areas (i.e., not from the IMPROVE network), it can be seen that the mean and median values are not markedly different, though in general $PM_{2.5}$ mass is about 50% of the mass of PM_{10}, which is then about 50% of the mass of TSP depending on the given area. As summarized in section 3.1.1, recent studies suggest that Pb is somewhat more likely to be found in fine fraction particles than in larger particle sizes. Overall, ambient air Pb concentrations in the United States are generally well below the current NAAQS level, except for a few scattered locations influenced by local sources.

Table 8-1. Descriptive Statistics for Lead Measurements (in µg/m^3) from Monitors* Using Different Size Fractions of PM for Recent Years**

Particle size (network)	Minimum	Mean	Median	Maximum
TSP*** (FRM, n~200)	0.00	0.01-0.22	0.02-0.04	1.92-9.13
TSP*** (FRM, n~200) excluding point source sites	0.00	0.03-0.05	0.01-0.02	0.26-1.75
PM_{10} (NATTS, n=26)	0.0027	0.0116	0.0101	0.039
$PM_{2.5}$ (Speciation, n=272)	0.002	0.008	0.005	0.168
$PM_{2.5}$ (IMPROVE, n=167)	0.0005	0.0016	0.0013	0.0065

* Excluding monitors representative of point source emissions.
** 2000-2004 for data from IMPROVE and TSP; 2002-2005 for data from the $PM_{2.5}$ speciation network and NATTS.
*** Data for TSP presented as range of values for each year.

8.2.4 Non-air Environmental Lead Exposure Routes

In addition to ambient air, major non-air environmental routes for exposure to Pb include: Pb in house dust; Pb-based paint in older homes; drinking water; and Pb-contaminated food. Lead exposure can also occur at times due to other idiosyncratic sources such as calcium supplements, Pb-based glazes, certain kinds of miniblinds, hair dye, and other consumer products that can widely vary in their prevalence and the potential risk posed by them.

Given the large amount of time people spend indoors, exposure to Pb in dusts and indoor air can be significant (see Section 3.2.3). For children, dust ingested via hand-to-mouth activity may be a more important source of Pb exposure than inhalation. However, dust can be resuspended through household activities, thereby posing an inhalation risk as well. A number of different sources can contribute to Pb in housedust, both from sources outside the home and from Pb-based paint.

Throughout early childhood, floor dust Pb contamination is a source of exposure. Lead-contaminated windowsill dust becomes an additional source of Pb intake during the second year of life when children stand upright. Because of normal mouthing behaviors and increased mobility, the highest blood Pb levels are seen in children between 18 and 36 months of age. This typically is observed after a rapid rise in blood Pb levels between 6 and 12 months. Even at low concentrations, Pb in housedust can have a notable effect on children's blood Pb levels.

For example, studies discussed in Section 3.2.3 show that, at a median floor dust Pb level of 5 $\mu g/ft^2$ (54 $\mu g/m^2$), ~5% of children had blood Pb levels $\geq$10 µg/dL. At a floor dust Pb loading of 50 $\mu g/ft^2$ (540 $\mu g/m^2$), the percentage of children with blood Pb levels $\geq$10 µg/dL rose to 20%. In another study, children exposed to floor dust Pb loadings in excess of 25 $\mu g/ft^2$ (270 $\mu g/m^2$) were at eight times greater risk of having blood Pb levels $\geq$10 µg/dL compared to children exposed to levels below 2.5 $\mu g/ft^2$ (27 $\mu g/m^2$).

Soil Pb is a significant contributor to elevated blood-Pb levels, especially among children, in populations residing near certain Superfund sites, as discussed in Section 3.2.2. For example, Pb levels in soil collected at residences near the Tar Creek Superfund Site (a Pb mining area in northeastern Oklahoma) reflected contamination by wind-dispersed mine wastes. More than 20% of residential soil samples exceeded the EPA action level of 500 ppm, and children's blood Pb levels tended to be higher in comparison to those of children living outside the Superfund towns. In this same area, blood-Pb levels were found to be highest among African-American, Mexican-American, and poor children. Blood-Pb levels were most commonly correlated with mean floor dust Pb loading and with soil Pb, especially front yard soil. Another study found that homes at the Jasper County Superfund Site in southwestern Missouri had significantly higher soil and dust Pb levels and significantly higher blood-Pb levels than areas outside of the Superfund site. There was a strong statistical relationship observed between blood-Pb levels and soil, dust, and paint Pb concentrations.

Lead-based paint was the most widely used, dominant form of house paint for many decades, and a significant percentage of homes (especially those built before 1978) still contain Pb-based paint on some surfaces, as discussed in Section 3.5.1. As Pb-based paint degrades, it becomes incorporated into house dust, as noted earlier in this chapter. Lead-based paint poses a potential exposure risk due to ingestion of Pb-contaminated dusts via normal hand-to-mouth activities and/or pica (which are common in children) or due to inhalation during renovation or demolition projects. Lead-based paint can pose a particularly serious inhalation risk for both adults and children during renovation activities that form easily inhaled Pb particles. The ingestion and/or inhalation of Pb derived from Pb-based paint has long been one of the most common causes of clinical Pb toxicity in the United States.

As discussed in Section 3.3, most U.S. drinking water distribution systems serving more than 3,000 people typically supply drinking water that meets the EPA tap water limit of

0.015 mg/L (15 ppb) set in 1991. Of 18 major U.S. cities illustrated in Table 3-11 of Chapter 3 as exceeding the EPA water Pb Action Level in the early 1990s, 14 had decreased their 90th percentile tap water concentrations to below the 15-ppb Action Level during recent monitoring periods (since the year 2000). On the other hand, with the introduction of chloramine as an alternative water treatment used in some cities across the United States, some increases in tap water-Pb concentrations have been detected in some municipal water supplies, raising concern about possible resultant increases in blood-Pb levels among affected water-use populations.

Very little Pb in drinking water comes from public water utility supplies per se, versus from leaded solder in plumbing or Pb in plumbing fixtures in buildings attached to utility distribution lines. Thus, Pb in drinking water occurs primarily as a result of corrosion from Pb pipes, Pb-based solder, or brass or bronze fixtures within a residence, as noted in Chapter 3. However, Pb in drinking water, although generally found at low concentrations in the United States, has been linked to elevated blood Pb concentrations in general population groups. In one U.S. prospective study, for example, children exposed to water with Pb concentrations >5 ppb had blood Pb levels ~1.0 µg/dL higher than children with water Pb levels <5 ppb (Lanphear et al., 2002). In another study of mothers and infants in Glasgow, Scotland, tap water was the main correlate of elevated maternal blood Pb levels (Watt et al., 1996). Thus, under certain conditions, water may not be a trivial source of Pb exposure in some locations.

Although marked reductions of Pb in U.S. market basket food supplies have occurred during the past several decades, Pb-contaminated food still can be an important route of Pb exposure (see Section 3.4). As shown in Table 8-2, dietary Pb intake levels for various U.S. general population groups have notably declined over recent decades, due largely both to reduced Pb air emissions from automotive gasoline as well as reduced use of solder in cans in the United States. Several recent studies in the U.S. and Australia indicate that daily dietary Pb intake is in the range of 2 to 10 µg/day (see Section 3.4). Lastly, some U.S. population groups that frequently consume canned foods imported from non-U.S. countries that still allow use of lead-soldered cans may be at distinctly greater risk for exposure to Pb via dietary intake and consequent higher blood-Pb concentration.

Table 8-2. Estimated Dietary Lead Intake in U.S. Population Groups in 1982-1984 versus 1994-1996.

Gender and Age Groups	1982-1984 Total Diet Study Dietary Pb intake (µg/day)	1994-1996 Total Diet Study Dietary Pb intake (µg/day) *
Infants, 6-11 months	16.7	0.8-5.7
Children, 2 years	23.0	2.4-10.1
Children, 6 years	–	3.5-13.2
Females, 14-16 years	28.7	3.6-14.9
Males, 14-16 years	41.3	4.0-17.1
Females, 20-25 years	29.6	3.5-15.6
Males, 20-25 years	40.9	4.2-18.8
Females, 60-65 years	30.4	3.6-16.0
Males, 60-65 years	37.6	4.5-19.5

*Note – dietary intakes presented as ranges based on use of different methods to account for measurements below limit of detection.

8.3 TOXICOKINETICS, BIOLOGICAL MARKERS, AND MODELS OF LEAD BURDEN IN HUMANS

Understanding the relationships between human exposure to Pb in external media (air, food, water, soil/dust) and internal Pb burden in blood and other body tissues is a key issue of much importance in carrying out risk assessments that evaluate the potential risk for adverse health effects to occur in response to various Pb exposure scenarios. Use of biomarkers to index Pb exposures is predicated on knowledge concerning Pb toxickinetics. Blood-Pb concentrations have long been the most widely used biomarker by which to index Pb exposures in children and adults (as discussed extensively in the 1977 Lead AQCD and the 1986 Lead AQCD/Addendum). At the time of the 1986 Lead AQCD, it was recognized that Pb distributed to and accumulated in several bone compartments which exhibited differing mobility profiles. It was also recognized that a larger fraction of total body burden of Pb is found in the bones of adults relative to children. The possibility of bone-Pb serving as a source of long-term internal Pb exposure was considered. New studies that have since been published on the kinetics of Pb movement into and out of bone demonstrate the importance of bone-Pb stores as a source of Pb to the blood in retired lead workers and during pregnancy, as discussed in Chapter 4 of this document.

Additional information regarding Pb absorption, distribution, and elimination in humans is also discussed in Chapter 4, and some of the most important points regarding these and other aspects related to Pb toxickinetics are summarized below.

8.3.1 Biokinetics of Lead Uptake and Internal Distribution

Humans are exposed to Pb mainly by ingestion and inhalation. The absorption of Pb is affected by factors such as an individual's age and diet, as well as chemical and physical properties of the ingested or inhaled Pb, as discussed in Section 4.2.1. Lead absorption appears to be increased by both iron and calcium deficiency. Fasting also increases the absorption of Pb from ingested soil. Lead absorption in humans may be a capacity limited process, such that the fraction of ingested Pb that is absorbed may decrease with increasing rate of Pb intake. The available studies to date, however, do not provide a firm basis for discerning whether the gastrointestinal absorption of Pb is limited by dose. The size of ingested Pb particles also affects absorption, with absorption decreasing as particle size increases.

In general, the Pb burden in the body may be viewed as being divided between a dominant slow compartment (bone) and a smaller fast compartment (soft tissues). This distribution of Pb in the body and factors affecting the exchange of Pb between bone and blood are discussed in detail in Sections 4.2.2, 4.3.1, and 4.3.2. In human adults, more than 90% of the total Pb body burden is found in the bones, whereas bone Pb accounts for ~70% of the body burden in children. The highest soft tissue concentrations in adults also occur in liver and kidney cortex. Lead in blood is exchanged between both of these compartments. The contribution of bone Pb to blood Pb changes with the duration and intensity of the Pb exposure, age, and various physiological variables (e.g., nutritional status, pregnancy, menopause).

As also discussed in Chapter 4, Pb accumulates in bone regions having the most active calcification at the time of exposure. Lead accumulation is thought to occur predominantly in trabecular bone during childhood and in both cortical and trabecular bone in adulthood. Lead concentrations in bone increase with age throughout life, indicative of a relatively slow turnover of Pb in adult bone. Lead content in some bones (i.e., mid femur and pelvic bone) increases into adulthood, plateaus at middle age, and then decreases at older ages. This decrease is most pronounced in postmenopausal females and may be due to osteoporosis and the release of Pb from resorbed bone to blood. Lead in adult bone can serve to maintain blood-Pb levels long after

external exposure has ceased. During pregnancy, bone Pb can also serve as a Pb source with the resorption of maternal bone for production of the fetal skeleton, and maternal bone Pb can continue postnatally to serve as a source of Pb exposure to the offspring via maternal lactation/breastfeeding (see Section 4.3.2.5).

In contrast to Pb in bone, which accumulates with continued exposure in adulthood, Pb concentrations in soft tissues (e.g., liver and kidney) are relatively constant in adults, reflecting a faster turnover of lead in soft tissue relative to bone (as discussed in Chapter 4). It is also noted that Pb in soft tissues exists predominantly bound to protein (see Section 5.11). High affinity cytosolic Pb-binding proteins (PbBPs) have been identified in rat kidney and brain. Other high-affinity Pb-binding proteins have been isolated in human kidney, two of which have been identified as a 5 kD peptide, thymosin 4, and a 9 kD peptide, acyl-CoA binding protein.

Lead in blood is found primarily (~99%) in the red blood cells. As discussed in Sections 4.2.2 and 4.3.1, δ-aminolevulinic acid dehydratase (ALAD) is the primary Pb-binding ligand in erythrocytes. Lead binding to ALAD is saturable; the binding capacity has been estimated to be ~850 μg/dL red blood cells (or ~340 μg/dL whole blood), with an apparent dissociation constant of ~1.5 μg/L. It has been suggested that the small fraction of Pb in plasma (<0.3%) may be the more biologically labile and toxicologically active fraction of circulating Pb. Several authors have proposed that Pb released from the skeleton was preferentially partitioned into serum compared with red cells. About 40 to 75% of Pb in the plasma is bound to proteins, of which albumin appears to be the dominant ligand. Lead in serum not bound to protein exists largely as complexes with low molecular weight sulfhydryl compounds (e.g., cysteine, homocysteine) and other ligands.

8.3.2 Selection of Blood-Lead Concentration as Key Index of Lead Exposure

Blood-Pb concentration is extensively used in epidemiologic studies as an index of exposure and body burden mainly due to the feasibility of incorporating its measurement into human studies relative to other potential dose indicators, e.g., lead in kidney, plasma, urine, or bone. Section 4.3.1 considers the use of blood Pb as a marker of Pb exposure and body burden, and the contribution of bone Pb to the blood is specifically discussed in Section 4.3.2.4. A single blood-Pb measurement may not distinguish between a history of long-term lower level Pb exposure from a history that includes higher acute exposures, as discussed by Mushak (1998).

An additional complication is that the relationship between Pb intake and blood-Pb concentration is curvilinear; that is, the increment in blood-Pb concentration per unit of Pb intake decreases with increasing blood-Pb concentration, both in children and in adults. In general, higher blood Pb concentrations can be interpreted as indicating higher exposures (or lead uptakes); however, they do not necessarily predict higher overall body burdens. Similar blood-Pb concentrations in two individuals (or populations) do not necessarily translate to similar body burdens or similar exposure histories. The disparity in the kinetics of blood Pb and cumulative body burden may have important implications for the interpretation of blood-Pb concentration measurements in some epidemiology studies, depending on the health outcome being evaluated.

Bone Pb, as also indicated in Chapter 4, has begun to be accorded increasing attention as another potentially useful marker for Pb exposure. It is thought that bone-Pb measurements likely constitute a better indication of overall past cumulative Pb exposure history than do blood Pb concentrations, which are more strongly influenced by recent Pb exposures. Approaches to measurement of bone Pb in living human or animal subjects are discussed in Section 4.3.2.2 and mainly involve different x-ray techniques that have undergone extensive intercomparison testing and refinements during the past decade or so. Still, in contrast to blood-Pb concentrations, bone-Pb measurements have not yet gained widespread use in epidemiologic studies as a key biomarker for Pb exposure.

In addition to blood Pb and/or bone Pb, concentrations of Pb in hair and urine have at times also been used as biomarkers of Pb exposure (see Sections 4.3.4 and 4.3.5). However, an empirical basis for interpreting hair Pb measures in terms of body burden or exposure has not been firmly established. As discussed in Chapter 4, hair Pb measurements are subject to error due to contamination of the hair surface with environmental Pb and contaminants in artificial hair treatments (e.g., dyeing, bleaching, permanents) and, as such, are a relatively poor predictor of blood-Pb concentration, particularly at low blood-Pb levels (<10 to 12 μg/dL). Spontaneous urine-Pb excretion also provides little reliable information, unless adjusted to account for unmeasured variability in urine flow rate. Analogous to blood-Pb concentration measurements, spontaneous urinary Pb excretion measured in an individual at a single point in time mainly reflects the recent exposure history. As a result, spontaneous urinary-Pb measurement may serve as a feasible surrogate for plasma-Pb concentration, and may be useful for exploring dose-response relationships for effect outcomes that may be more strongly associated with plasma-Pb

level than overall Pb body burden. On the other hand, measurement of notably increased urinary Pb excretion in response to Succimer or other approved chelant challenge has proven to be reliable in both pediatric clinical and occupational settings as reflecting a history of excessive Pb exposure.

8.3.3 Trends in U.S. Blood Lead Levels

As discussed in Section 4.3.1.3, blood-Pb concentrations in the U.S. general population have been monitored over the past three decades via the National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control and Prevention. Data from the most recent survey (NHANES IV, Centers for Disease Control, 2005) are shown in Tables 8-3 and 8-4. For survey years 2001-2002, the geometric mean blood-Pb level for ages >1 year (n = 8,945) was 1.45 µg/dL (95% CI: 1.39, 1.52); with the geometric mean in males (n = 4,339) being 1.78 µg/dL (95% CI: 1.71, 1.86) and in females (n = 4,606) being 1.19 µg/dL (95% CI: 1.14, 1.25). Blood-Pb concentrations in the U.S. general population have decreased over the past three decades as regulations regarding leaded fuels, leaded paint, and lead-containing plumbing materials have decreased Pb exposure among the general population. Changes in average blood-Pb concentrations among U.S. children over time are shown in Figure 8-5.

Blood Pb concentrations can vary considerably as a function of age, physiological state (e.g., pregnancy, lactation, menopause), and numerous other factors that affect exposure to Pb. The NHANES data provide estimates for average blood lead concentrations in various demographic strata of the U.S. population. NHANES III Phase 2 samples were collected during 1991 to 1994. Geometric mean blood-Pb concentrations of U.S. adults, ages 20 to 49 years, estimated from the NHANES III Phase 2, were 2.1 µg/dL (95% CI, 2.0, 2.2). Among adults, blood-Pb concentrations were highest in the strata that included ages 70 years and older (3.4 µg/dL; 95% CI, 3.3, 3.6). The geometric mean blood-Pb concentration of children, ages 1 to 5 years, was 2.7 (95% CI, 2.5, 3.0) for the 1991 to 1994 survey period; however, the mean varied with socioeconomic (SES) status and other demographic characteristics that have been linked to Pb exposure (e.g., age of housing). Central estimates from the NHANES III Phase 2 (1991 to 1994), when compared to those from NHANES III Phase 1 (1988 to 1991) and the NHANES II (1976 to 1980), indicate a clear downward temporal trend in U.S. blood-Pb concentrations over

Table 8-3. Blood Lead Concentrations in United States by Age, NHANES IV (1999–2002)

Age	1–5 years		6–11 years		12–19 years		≥20 years	
Survey Period	*1999–2000*	*2001–2002*	*1999–2000*	*2001–2002*	*1999–2000*	*2001–2002*	*1999–2000*	*2001–2002*
N	723	898	909	1,044	2,135	2,231	4,207	4,772
Blood Lead (µg/dL)[a]	2.23 (1.96, 2.53)	1.70 (1.55, 1.87)	1.51 (1.36, 1.66)	1.25 (1.14, 1.36)	1.10 (1.04, 1.17)	0.94 (0.90, 0.99)	1.75 (1.68, 1.81)	1.56 (1.49, 1.62)

[a]Blood lead concentrations presented are geometric means (95% CI).

Table 8-4. Blood Lead Concentrations in United States by Gender, NHANES IV (1999–2002)

Gender	Males		Females	
Survey Period	1999–2000	2001–2002	1999–2000	2001–2002
n	3,913	4,339	4,057	4,606
Blood Lead (µg/dL)[a]	2.01 (1.93, 2.09)	1.78 (1.71, 1.86)	1.37 (1.32, 1.43)	1.19 (1.14, 1.25)

[a]Blood lead concentrations presented are geometric means (95% CI).

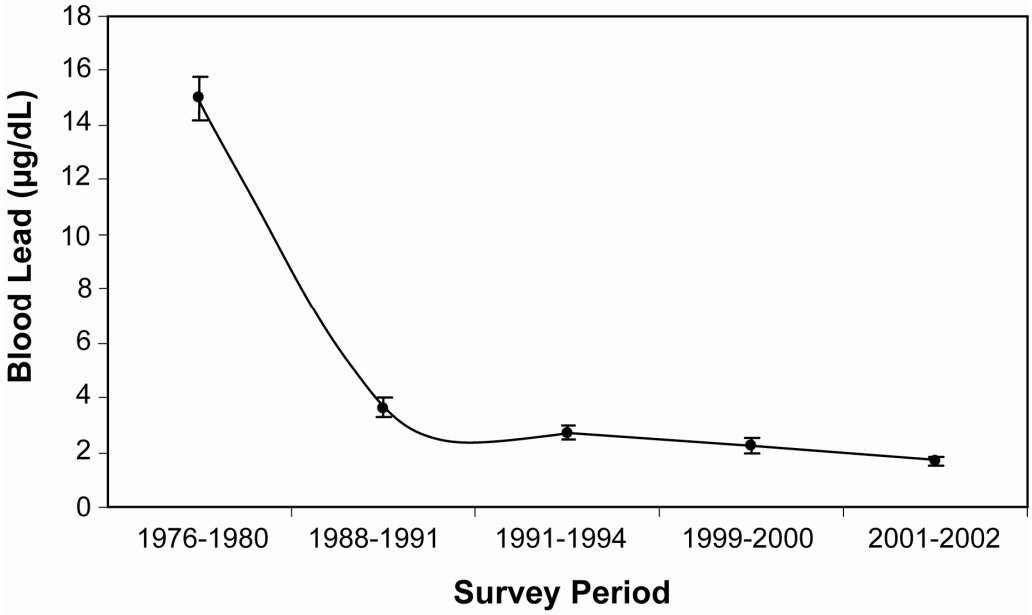


Figure 8-5. Blood lead concentrations in U.S. children, 1-5 years of age. Shown are geometric means and 95% confidence intervals as reported from the NHANES II (1976–1980) and NHANES III Phase 1 (1988–1991; Pirkle et al., 1994); NHANES III Phase 2 (1991–1994; Pirkle et al., 1998); and NHANES IV (1999-2000, 2001-2002; Centers for Disease Control, 2005).

the past 20 years or so. It should be noted, however, that blood-Pb levels have been declining at differential rates for various general subpopulations, as a function of income, race, and certain other demographic indicators such as age of housing. Also, substantial caution should be exercised with regard to use of NHANES data for risk assessment purposes, in that the nationally-representative NHANES quantitative results (e.g., national mean blood-Pb levels or strata-classified national mean blood-Pb values) vary with regard to how reflective they may be of specific regions or communities.

8.3.4 Approaches to Predictive Estimation of Pb-Exposure Impacts on Distribution to Internal Tissues

As indicated in Chapter 4, a key issue of much importance in carrying out lead risk assessments that evaluate the potential likelihood of Pb-induced health effects is the estimation of external Pb-exposure impacts on internal Pb tissue concentrations. This includes estimation of

typical Pb exposure impacts on internal distribution of Pb to blood and bone (as key biomarkers of Pb exposure), as well as to other "soft tissue" target organs (e.g., brain, kidney, etc.). Earlier criteria assessments in the 1977 and 1986 Lead AQCDs extensively discussed then available slope factor and/or other regression models of external Pb exposure impacts on blood-Pb concentrations in human adults and children. The older slope factor analyses discussed in the 1977 and 1986 Lead AQCDs noted that at relatively low air-Pb concentrations (≤ 2 µg/m^3), pediatric blood-Pb levels generally increase by ~2 µg/dL per each 1 µg/m^3 increment in air-Pb concentration. Further refinements in regression modeling of Pb impacts on blood or bone Pb are discussed in Chapter 4.

Several new studies discussed in Chapter 4 have investigated relationships between Pb exposure and blood Pb in children (see Section 4.4.2). These studies support the concept that contact with Pb in surface dust (interior and exterior) is a major contributor to Pb intake in children. In one meta-analysis, the most common exposure pathway to emerge as notably influencing blood-Pb concentration was exterior soil, operating through its effect on interior dust Pb and hand Pb. Using a structural equation model, other analyses also found that the exposure pathway component that was most influential on blood Pb was interior dust Pb loading, directly or through its influence on hand Pb. Both soil and paint Pb influenced interior dust Pb. However, interior and exterior paints were more significant contributors to childrens' blood-Pb levels in urban (heavily paint-impacted) areas than at western U.S. extractive (mining/smelting) industry sites, and dust Pb was more significantly linked to soil Pb than paint Pb at such western sites. Still, these and other studies of populations near active sources of air emissions (e.g., smelters, etc.), substantiate the effect of airborne Pb and resuspended soil Pb on interior dust and blood Pb.

Both exterior soil and paint Pb contribute to interior dust Pb levels. It has been estimated that for every 1000 ppm increase in soil-Pb concentration, pediatric blood-Pb levels generally increase by ~1 to 5 µg/dL in exposed infants and children <6 years old. All ingested lead is not absorbed to the same extent, in that intake of soil-Pb with low bioaccessibility or bioavailability characteristics can yield distinctly lower-than-typical blood-Pb increments. Factors such as an individual's age and diet, as well as chemical and physical properties of Pb, affect absorption, e.g., absorption is increased by fasting and dietary iron or calcium deficiencies.

Additional information on Pb biokinetics, bone mineral metabolism, and Pb exposures has led to refinements and expansions of earlier modeling efforts. In particular, there are three pharmacokinetic models that are currently being used or considered for broad application in Pb risk assessment: (1) the Integrated Exposure Uptake BioKinetic (IEUBK) model for Pb in children developed by EPA (U.S. Environmental Protection Agency, 1994a,b; White et al., 1998); (2) the Leggett model, which also simulates Pb kinetics from birth through adulthood (Leggett, 1993); and (3) the O'Flaherty model, which simulates Pb kinetics from birth through adulthood (O'Flaherty, 1993, 1995). The above three models have been individually evaluated to varying degrees, against empirical physiological data on animals and humans and data on blood-Pb concentrations in individuals and/or populations (U.S. Environmental Protection Agency, 1994a,b; Leggett, 1993; O'Flaherty, 1993). In evaluating models for use in risk assessment, exposure data collected at hazardous waste sites have mainly been used as inputs to model simulations (Bowers and Mattuck, 2001; Hogan et al., 1998). The exposure module in the IEUBK model makes this type of evaluation feasible. Exposure-biokinetics models both illustrate exposure-blood-body burden relationships and provide a means for making predictions about these relationships that can be experimentally or epidemiologically tested.

The EPA IEUBK model for Pb has gained widespread use for risk assessment purposes in the United States, and it is currently clearly the model of choice in evaluating multimedia Pb exposure impacts on blood-Pb levels and distribution of Pb to bone and other tissues in young children <7 years old. The EPA All Ages Lead Model (AALM), now under development, aims to extend beyond IEUBK capabilities to model external Pb exposure impacts (including over many years) on internal Pb distribution not only in young children, but also in older children, adolescents, young adults, and other adults well into older years (up to 90 years of age). The AALM essentially uses adaptations of IEUBK exposure module features, coupled with adaptations of IEUBK biokinetics components (for young children) and of Leggett model biokinetics components (for older children and adults). However, the AALM has not yet undergone sufficient development and validation for it to be recommended for general risk assessment use.

8.4 LEAD-INDUCED TOXICITY: INTEGRATION OF TOXICOLOGIC AND EPIDEMIOLOGIC EVIDENCE

8.4.1 Introduction

As discussed in the previous two chapters (Chapters 5 and 6) dealing with the toxicology and epidemiology of Pb-induced health effects, Pb has been shown to exert a broad array of deleterious effects on multiple organ systems via widely diverse mechanisms of action. Truly remarkable progress has been made during the past several decades with regard to (a) more fully delineating over time the wide variety of pathophysiologic effects associated with Pb exposure of human population groups and laboratory animals and (b) the characterization of applicable exposure durations and dose-response relationships for the induction of the multifaceted Pb effects. This progress has been well documented by the previous Pb NAAQS criteria reviews carried out by EPA in the late 1970s and during the 1980s, as well as being well reflected by previous chapters of this document.

The 1977 Lead AQCD (U.S. Environmental Protection Agency, 1977) that provided key scientific bases for the setting in 1978 of the current Pb NAAQS included discussion of both: (a) historical literature accumulated during several preceding decades that established Pb encephalopathy and other signs and symptoms of persisting severe central and/or peripheral nervous system damage, as well as renal and hepatic damage, and anemia as typifying the classic syndrome of acute and/or chronic high-level Pb poisoning among human pediatric and /or adult population groups, and (b) evaluation of then newly-emerging evidence for more subtle and difficult-to-detect "subclinical" Pb effects on IQ, other neurological endpoints, and moderate blood hemoglobin deficits or other erythropoietic indicators of heme synthesis impairment, which collectively were judged to constitute an array of adverse Pb health effects associated with Pb exposures indexed by blood Pb concentrations ranging down to ~30 µg/dL. The next Pb NAAQS criteria review during the 1980's, as contained in the 1986 Lead AQCD/Addendum and its 1990 Supplement (U.S. Environmental Protection Agency, 1986a, b, 1990) documented further rapid advances in Pb health effects research that provided (a) increasingly stronger evidence that substantiated still lower fetal and/or postnatal Pb-exposure levels (indexed by blood-Pb levels extending to as low as 10 to 15 µg/dL or, possibly, below) as being associated with slowed physical and neurobehavioral development, lower IQ, impaired learning, and/or other indicators of adverse neurological impacts and (b) other pathophysiological effects of Pb

on cardiovascular function, immune system components, calcium and vitamin D metabolism, and other selected health endpoints.

Newly available scientific information published since the 1986 Lead AQCD/Addendum and the 1990 Supplement, as assessed in previous chapters of this document, further expands our understanding of a wide array of Pb-induced health effects, underlying mechanisms, and factors that enhance or lessen susceptibility to Pb effects. Very importantly, the newly available toxicologic and epidemiologic information, as integrated below, includes assessment of new evidence substantiating risks of deleterious effects on certain health endpoints being induced by distinctly lower than previously demonstrated Pb exposures indexed by blood-Pb levels extending well below 10 µg/dL in children and/or adults.

The ensuing subsections provide concise summarization and integrative synthesis of the most salient health-related findings and conclusions derived from the current criteria assessment. This includes discussion of new toxicologic and/or epidemiologic evidence concerning Pb-induced (a) effects on neurobehavioral development and other indicators of nervous system effects; (b) cardiovascular effects; (c) heme synthesis effects; (d) renal effects; (e) immune system functions; (f) effects on calcium and vitamin D metabolism; (g) inter-relationships to bone and teeth formation and demineralization; (h) effects on reproduction and other neuroendocrine effects; and (i) genotoxicity and carcinogenic effects.

8.4.2 Neurotoxic Effects

The neurotoxic effects of Pb exposure are among those most studied and most extensively documented among human population groups. Also, extensive experimental laboratory animal evidence has been generated that (a) substantiates well the plausibility of the epidemiologic findings observed in human children and adults and (b) expands our understanding of likely mechanisms underlying the neurotoxic effects. Two major issues are important in considering the concordance of human and animal results: (1) comparability of blood Pb levels (or other internal dose markers) among species; and (2) comparability of neurobehavioral tests for animals and humans.

Animal models are extremely important in the characterization of Pb neurotoxicity, because exposures can be controlled to address questions about sensitive periods of exposure. Unlike typical human exposures reported in epidemiology studies, Pb dosing to animals can be

stopped at any time to address questions about the reversibility and persistence of neurotoxic effects. Also, with animals, dosing can be varied to include very low doses to examine effects seen with more current pediatric exposures. Animal models, especially inbred strains of rodents, can lessen the effects of the critical confounder of parental cognitive ability, which parallels human IQ. Also eliminated in controlled animal exposures are the confounders of SES and nutrition.

In a review, Davis et al. (1990) state that little effort has been directed toward making direct comparisons of human and animal dose-response relationships because of the abundance of human exposure-effect data. The 1986 Lead AQCD also reported that there exists some uncertainty in extrapolating from animals to humans because blood-Pb levels may not be directly comparable. Both rats and monkeys may require higher Pb exposure levels than humans to achieve a comparable blood-Pb level. It was further recognized by Davis et al. (1990) that, due to inadequate numbers of subjects and the resulting lack of statistical power, it may not be possible to detect subtle Pb-induced neurotoxic effects in both epidemiologic and experimental studies.

As discussed in the 1986 Lead AQCD, questions have also been raised regarding the comparability between neurobehavioral effects in animals and effects on human behavior and cognitive function. One major difficulty is the lack of standardized methodologies or a consistent operational definition by which to compare behavioral endpoints. In addition, behavior is difficult to compare meaningfully across species, because behavioral analogies do not necessarily demonstrate behavioral homologies. Davis et al. (1990) examined the comparative neurotoxicity of Pb in humans and animals and noted that a problem in comparing behavior and identifying behavioral similarities is that behavior is not a phenomenological given, but an event or series of events that must be represented by abstracting of one or more of its features. They further state that it is important of be mindful of "the degree to which the model faithfully reflects the mechanisms underlying its referent."

In assessing the comparability of measures of cognitive function in humans and animals, Sharbaugh et al. (2003) also state that of ultimate importance is finding sensitive homologous or parallel neurobehavioral tests in humans and animals. Homologous tests are those for which the same procedure is followed in humans and the animal species. Examples of homologous tests include Bayley Scales of Infant Development II, which tests a number of behavioral and reflect

tasks, and the visual recognition memory test. Both tests are performed in human infants and nonhuman primates. Parallel tests are those that are conducted in a different manner in humans and animals, but for which it is believed that the same cognitive function is being measured, e.g., tests of learning, recognition memory, and long-term memory in humans and rodents. Generally measures of cognitive function for humans and nonhuman primates are homologous, while those with rodents are parallel (Sharbaugh et al., 2003).

The most widely used measure of cognitive function in epidemiologic studies is the intelligence quotient or IQ score. An IQ score is a global measure reflecting the integration of numerous behavioral processes. There is no direct parallel to IQ tests for nonhuman primates or rodents. However, in animals a wide variety of tests that assess attention, learning, and memory suggests that Pb exposure results in a global deficit in functioning, just as it is indicated by decrements in IQ scores in children (Rice, 1996).

Examination of the effect of Pb on behavioral processes in human and experimental animals needs to focus beyond IQ, as noted by Cory-Slechta (1996). One strategy would be to use the same behavioral baselines in human studies that have revealed Pb-related deficits in cognitive functions in experimental animal studies, particularly those such as discrimination learning, reversal learning, repeated learning of response sequences, and concurrent schedule transitions. Rice (1996) concurs with this view and states further that the use of IQ has proven to be a sensitive indicator of Pb exposure, but that using more specific tests could provide even greater sensitivity. In the following sections, the epidemiologic and toxicologic evidence of Pb-induced effects on global as well as specific neurobehavioral outcomes are integrated and discussed.

8.4.2.1 Neurocognitive Ability

Global Measures of Cognitive Function – Intelligence Testing and Academic Achievement

Lead effects on human neurocognitive ability have been assessed in epidemiologic studies largely by use of age-appropriate, standardized IQ tests (as discussed in Section 6.2.3 of Chapter 6). Assessment of intelligence in infants and young children has been performed using a number of scales, including the various Bayley Scales of Infant Development and the McCarthy Scales of Children's Abilities. Most studies used the Weschler Intelligence Scales for Children-Revised (WISC-R) in older children. As discussed by Rice (1996), it is generally recognized

that early tests of intelligence such as the Bayley scales do not measure the same functions as tests used at school age such as the WISC-R and have little predictive validity for individual children (though the Bayley scales may have better predictive power for low-functioning children). Regardless, numerous well-conducted longitudinal cohort and cross-sectional studies that evaluated various study populations in several different countries have consistently found Pb-related IQ deficits from infancy through at least early school age.

For example, in the largest available new cross-sectional study, Lanphear et al. (2000) examined the relationship between blood Pb concentrations and cognitive deficits in a nationally representative sample of 4,853 U.S. children aged 6 to 16 years (geometric mean blood Pb of 1.9 µg/dL) who participated in NHANES III, with 97.9% of the children having blood-Pb concentrations <10 µg/dL. Two subtests of the WISC-R, Block Design (a measure of visual-spatial skills) and Digit Span (a measure of short-term and working memory) were administered; and numerous potential confounders were assessed in the multivariable analyses. Although no data on maternal IQ or direct observations of caretaking quality in the home were available, other variables such as the poverty index ratio and education level of the primary caregiver may have served as adequate surrogate measures of these important potential confounders. In multivariate analyses, a significant covariate-adjusted relationship was found between blood-Pb level and scores on both WISC-R subtest for all children as well as among those with blood-Pb levels <10 µg/dL. Blood-Pb concentration was also significantly associated with Block Design when the multivariate analysis was restricted to children with blood Pb levels <7.5 µg/dL.

Other recent studies of the association of Pb with IQ in children with low Pb exposures have consistently observed effects at blood-Pb concentrations below 10 µg/dL (as discussed in Section 6.2.3 of Chapter 6). Most notably, a large international pooled analysis of 1,333 children from seven different cohorts by Lanphear et al. (2005) estimated a decline of 6.2 points (95% CI: 3.8, 8.6) in full scale IQ for an increase in concurrent blood-Pb level from 1 to 10 µg/dL. A common observation among some of these studies of low-level Pb exposure is a non-linear dose-response relationship between blood Pb and neurodevelopmental outcomes. Although this may seem at odds with certain fundamental toxicological concepts, it is possible that the initial neurodevelopmental lesions seen at lower Pb levels may be disrupting different biological mechanisms (e.g., early developmental processes in the central nervous system) than the more severe effects of high Pb exposures that result in symptomatic poisoning and frank mental

retardation. One ad hoc explanation may be that the predominant mechanism at very low blood-Pb levels is rapidly saturated and that a different, less-rapidly-saturated process, becomes predominant at blood-Pb levels greater than 10 µg/dL.

Another global measure of cognitive function is academic achievement. Compared to the vast number of studies assessing the blood Pb-IQ relationship in children, there are relatively little data available on the relationship between Pb exposure and objective measures of academic achievement. These studies focused on the effect of Pb on school performance, including reading, math, spelling, and handwriting (see Section 6.2.4).

Lanphear et al. (2000) examined the relationship between blood-Pb levels and a standardized measure of academic achievement among 4,853 NHANES III children, aged 6 to 16 years (geometric mean blood-Pb of 1.9 µg/dL). Subjects were administered the Arithmetic and Reading subtests of the Wide Range Achievement Test-Revised (WRAT-R). Multiple linear regression revealed significant Pb-related decrements in Arithmetic and Reading scores. In analyses stratified by blood-Pb levels, statistically significant inverse relationships between blood-Pb levels and performance for both Reading and Arithmetic subtests were found for children with concurrent blood-Pb concentrations <5 µg/dL. However, possible attribution of the observed associations of decrements in WRAT-R scores to earlier (but unmeasured) likely somewhat higher peak blood-Pb concentrations cannot be ruled out.

Several other epidemiologic studies observed inverse associations between exposure to Pb and academic achievement, for the endpoints noted above as well as class rankings and high school graduation rates. Two studies specifically examined the effects of blood-Pb levels <10 µg/dL on academic achievement. One study examined 533 girls aged 6 to 12 years (mean blood Pb level of 8.1 µg/dL) in Riyadh, Saudi Arabia and observed that, in a subset of students with blood-Pb levels <10 µg/dL, class rank percentile was statistically significantly associated with blood-Pb levels (Al-Saleh et al., 2001). In another study in Torreón, Mexico, a significant inverse relationship was found between blood-Pb concentrations and math and vocabulary scores in 594 second graders (mean blood Pb of 11.4 µg/dL). In segmented regression analyses, slopes for blood Pb associations with vocabulary and math scores were significantly steeper below 10 µg/dL than above (Téllez-Rojo et al., 2006). Associations between Pb exposure and academic achievement observed in the above-noted studies were significant even after adjusting for IQ, suggesting that Pb-sensitive neuropsychological processing and learning factors

not reflected by global intelligence indices might contribute to reduced performance on academic tasks.

Specific Cognitive Abilities – Learning, Memory, and Attention

In addition to IQ and academic achievement, epidemiologic studies have evaluated Pb effects on specific cognitive abilities, e.g., attention, executive functions, language, memory, learning, and visuospatial processing. Results from these studies are most comparable to those experimental animal studies examining Pb effects on learning ability, memory, and attention.

Executive functions refer to an individual's ability to regulate attention and engage several related higher order cognitive processes such as strategic planning, control of impulses, organized search, flexibility of thought and action, and self-monitoring of one's own behavior. In some earlier studies, assessed in the 1986 Lead AQCD/Addendum and/or 1990 Supplement, Pb exposure was associated with higher frequency of negative ratings by teachers and/or parents on behaviors such as inattentiveness, impulsivity, distractibility, and lack of persistence on assigned tasks, as well as slowed psychomotor responses and more errors on simple, serial, and choice reaction time tasks. More recent studies (see Section 6.2.5) have observed inverse relationships between exposure to Pb and attentional behaviors and executive function, even in cohorts where more than 80% of the children had blood-Pb levels <10 μg/dL. These associations were observed across a wide range of age groups, from children 4-5 years to 19-20 years of age. Higher blood-Pb levels were also associated with impaired memory and visual-spatial skills.

Whether the domains of executive functions, attention, memory, or visual-motor integration per se are specifically sensitive to Pb is unknown, as there is rarely a one-to-one correspondence between performance on a focused neuropsychological test and an underlying neuropsychological process. For example, a low score on the visual-motor integration test may reflect singular or multiple neurobehavioral deficits, e.g., difficulties with graphomotor control, visual perception, behavioral monitoring (impulsivity), and/or planning (executive functions). Early Pb exposure may be associated with poorer performance on executive/regulatory functions that are thought to depend on the frontal or prefrontal brain regions. The prefrontal cortex is highly innervated by neuronal projections from the midbrain and has the highest concentration of dopamine of all cortical areas. The dopamine system, which plays a key role in cognitive

abilities mediated by the prefrontal cortex, is particularly sensitive to Pb, based on data from studies of rodents and nonhuman primates (see Section 5.3). These animal toxicology findings provide strong biological plausibility in support of the concept that Pb may impact one or more of these specific cognitive functions in humans.

Results from fixed interval (FI) studies in 4 species of laboratory animal models at environmentally relevant doses (as shown in Figure 5-6) clearly demonstrate that Pb induces increased response rates. The increased response rates are mostly due to shortened time to initiate responding in the interval and more rapid response once the responding begins. This pattern of effects has been compared to young human males diagnosed with Attention Deficit/Hyperactivity Disorder (ADHD), and it is thought that increases in response rates found in animal models parallel increases in impulsivity in self-control paradigms (as noted by Cory-Slecta, 2003a).

As noted in Section 5.3.5, NMDAR function and ontogeny are affected by Pb exposure. Functional NMDARs are necessary for spatial learning and memory, as tested by the Morris water maze. Several studies that evaluated Pb effects with this learning paradigm have shown that chronic exposure to 250 ppm Pb affected long-term memory. The effect of Pb on memory is not clearly understood. In some studies, memory impairment was found at blood Pb levels of 10 µg/dL, whereas numerous other studies found no Pb-induced effects on short term memory. This parallels findings from most cross-sectional and prospective epidemiological studies, which generally did not detect low-level Pb exposure effects on memory.

Studies of early developmental cognitive ability in monkeys postnatally exposed to Pb (see Section 5.3.5) have used the Early Infant Behavioral Scale, which is modeled after the Brazelton Neonatal Behavioral Assessment. The monkeys displayed both decreased visual attentiveness and increased agitation. Other epidemiologic studies using the Brazelton scale have shown analogous results for human infants.

8.4.2.2 Behavior, Mood, and Social Conduct

Investigating associations between Pb exposure and behavior, mood, and social conduct of children has been an emerging area of research (see Section 6.2.6). Early studies indicated linkages between lower-level Pb toxicity and behavioral problems (e.g., aggression, attentional problems, and hyperactivity) in children. Blood-Pb and tooth-Pb levels have been associated

with behavioral features of ADHD, including distractibility, poor organization, lacking persistence in completing tasks, and daydreaming, in various cohorts of children with a wide range of Pb exposures. In the Port Pirie, Australia cohort study, the relationship between Pb exposure and emotional and behavioral problems at ages 11 to 13 years were examined after stratifying the data set by gender. Stronger associations with Pb were observed for externalizing behavior problems in boys compared to girls. In contrast, greater internalizing behavior problems were observed for girls than in boys.

The relationship between Pb exposure and delinquent and criminal behavior also has been addressed in several investigations. Studies linking attention deficits, aggressive and disruptive behaviors, and poor self-regulation with Pb have raised the prospect that early exposure may result in an increased likelihood of engaging in antisocial behaviors in later life. In two prospective cohort studies conducted in Pittsburgh (Needleman et al., 1996) and Cincinnati (Dietrich et al., 2001), elevated Pb levels were associated with several measures of behavioral disturbance and delinquent behavior. It was also observed that bone-Pb levels in adjudicated delinquents were significantly higher than in non-delinquent community control subjects in Pittsburgh and surrounding Allegheny County, PA environs. In a Philadelphia survey of 987 African-American youths, a history of Pb poisoning was among the most significant predictors of delinquency and adult criminality in males (Denno, 1990).

These results indicate that Pb may play a role in the epigenesis of behavioral problems in inner-city children independent of other social and biomedical cofactors. The particular biological mechanisms that may underlie Pb effects on aggression, impulsivity, and poor self-regulation are not yet well understood. However, Pb impacts many brain sites and processes involved in impulse control (Lidsky and Schneider, 2003). Also, the increased risk of delinquency may indirectly be a consequence of attentional problems and academic under-achievement among children who suffered higher Pb exposures during their formative years (as noted by Needleman et al., 2002).

Lead has been shown to affect reactivity to the environment and social behavior in both rodents and nonhuman primates at blood-Pb levels of 15 to 40 μg/dL, though the literature has some conflicting studies (see Section 5.3.5). In general, most studies show a Pb-induced enhancement of social investigation and exploratory behavior. Aggression was increased in hamsters, but not in rats, though the latter did display increased behavioral reactivity to stimuli.

Early postnatal testing of Pb-exposed rhesus monkeys has shown lowered muscle tonus, greater agitation, and decreased visual attentiveness. Chronically exposed rhesus monkeys exhibited Pb-induced disruption of social play and increased self-stimulation and fearful behavior that persisted for months after exposure ended. Thus, no clear pattern is yet apparent in the experimental literature examining aggression that parallels the epidemiologic findings of Pb-induced increases in aggression and delinquent behavior among humans. However, the findings of increased reactivity to stimuli, impulsivity, and attention dysfunction found in both Pb-exposed animals and humans may underlie some of the behavioral and emotional problems observed epidemiologically.

8.4.2.3 Neurophysiologic Outcomes

Epidemiologic studies of the effect of Pb on sensory acuity have focused on hearing thresholds and features of auditory processing in Pb-exposed children (see Section 6.2.7). Schwartz and Otto (1987) observed significant Pb-associated elevations in pure-tone hearing thresholds at various frequencies within the range of human speech among over 4,500 subjects (4 to 19 years old) in NHANES II. These findings were replicated in a sample of ~3,000 subjects (6 to 19 years old) in the Hispanic Health and Nutrition Examination Survey (HHANES) (Schwartz and Otto, 1991), including at blood-Pb levels <10 µg/dL.

Dietrich et al. (1992) assessed the relationship between scores on a test of central auditory processing (SCAN) and blood-Pb concentrations in 215 children 5 years of age drawn from the Cincinnati Lead Study. Higher prenatal, neonatal, and postnatal blood-Pb concentrations were associated with more incorrect identification of common monosyllabic words presented under conditions of filtering (muffling). In another study, conducted in Poland, a significant association between concurrent blood-Pb levels and increased hearing thresholds was also observed among 155 children 4 to 14 years of age (median blood Pb of 7.2 µg/dL) (Osman et al., 1999). This relationship remained statistically significant when restricted to children with blood-Pb levels <10 µg/dL. The supportive evidence of a relationship between Pb exposure and auditory processing suggests that Pb-related deficits in hearing and auditory processing may be one plausible mechanism by which an increased Pb burden might impede a child's learning (Bellinger, 1995).

Animal studies have shown Pb-induced deficits in both auditory and visual acuity, which may contribute to the cognitive deficits associated with Pb exposure. Blood Pb levels as low as 33 μg/dL in nonhuman primates impair auditory function by increasing latencies in brainstem auditory evoked potentials and elevating hearing thresholds. Blood-Pb levels of 19 μg/dL in rats have been found to cause selective effects on rod and bipolar cells, resulting in decreased maximal ERG amplitude, decreased ERG sensitivity, and increased mean ERG latency. In a review of Pb-induced auditory and visual dysfunction, Otto and Fox (1993) point to the structural, biophysical, and photochemical similarities of rods in rats, monkeys and humans and suggest that undetected visual or auditory deficits may profoundly impact both sensory motor and mental development in children.

Electrophysiological evaluations have been conducted on Pb-exposed children in attempts to obtain a more direct measure of the toxicant's impact on the nervous system (as discussed in Section 6.2.9). Much of this work was conducted by Otto and colleagues during the 1980s and demonstrated effects of Pb on neurosensory functioning (auditory and visual evoked potentials) across a broad range of exposures. A more recent study examining the associations between Pb exposure and brainstem auditory evoked responses observed results that were less consistent (Rothenberg et al., 1994).

The methods of Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) have also been applied to evaluate Pb-exposed children. Several studies compared subjects with elevated blood-Pb levels (blood Pb ≥23 μg/dL) to control subjects (blood Pb <10 μg/dL). Although all of the participants had normal MRI examinations, the Pb-exposed subjects exhibited a significant reduction in the ratios of N-acetylaspartate to creatine and phosphocreatine in frontal gray matter compared to controls (Trope et al., 2001). Similarly, reduced peak values of N-acetylaspartate, choline, and creatine were found in all four brain regions in Pb-exposed children relative to control subjects (Meng et al., 2005).
The observed reductions in brain N-acetylaspartate levels may be related to decreased neuronal density or neuronal loss. Also, reduced choline signal may indicate decreased cell membrane turnover or myelin alterations that could lead to central nervous system hypertrophy, while lower creatine may indicate reduced neuronal cell viability.

Using functional MRI (fMRI), a subsample of 48 young adults (aged 20-23 years) from the Cincinnati Lead Study performed an integrated verb generation/finger tapping paradigm

(Cecil et al., 2005; Yuan et al., 2006). Higher childhood average blood-Pb levels were significantly associated with reduced activation in Broca's area, a recognized region of speech production in the left hemisphere, and increased activation in the right temporal lobe, the homologue of Wernicke's area (an area associated with speech production) in the left hemisphere. This suggests that elevated childhood Pb exposure may influence neural substrates underlying semantic language function in normal language areas, with concomitant recruitment of contra-lateral regions causing a dose-dependent atypical organization of language function.

8.4.2.4 Neuromotor Function and Vocalization

Only a few recent epidemiologic studies have evaluated neuromotor deficits as an outcome of early Pb exposure (see Section 6.2.8). In the Cincinnati Lead Study cohort, blood-Pb levels, both neonatal and postnatal, were significantly associated with poorer scores on measures of bilateral coordination, visual-motor control, upper-limb speed and dexterity, fine motor composite from the Bruininks-Oseretsky scales, and postural stability in children 6 years of age (Dietrich et al., 1993b). In general, the strongest and most consistent relationships were observed with concurrent blood-Pb levels (mean 10.1 μg/dL). At 16 years of age, 78-month postnatal blood-Pb levels were significantly associated with poorer fine-motor skills, as indexed by covariate-adjusted factor scores derived from a factor analysis of a comprehensive neuropsychological battery. Variables loading highly on the fine-motor component came from grooved pegboard and finger tapping tasks. In the Yugloslavian Prospective Study, lifetime average blood-Pb concentration through 54 months of age was associated with poorer fine motor and visual motor function, but was unrelated to gross motor function (Wasserman et al., 2000a).

Another recent study examined the effect of multiple exposures (including Pb, mercury, and PCBs) on neuromotor functions in 110 preschool Inuit children residing in Canada (Després et al., 2005). Significant associations that were found only for blood-Pb concentrations (mean of 5.0 μg/dL) were those associated with increased reaction time, sway oscillations, alternating arm movements, and action tremor. Even after eliminating children with blood-Pb levels >10 μg/dL (10% of cohort) from the analyses, results generally remained consistent, suggesting that neuromotor effects of Pb occurred at blood Pb levels <10 μg/dL.

Changes in vocalization are a potential biomarker for Pb exposure. That is, analyses of acoustical cries in babies showed that percent nasalization decreased progressively over cord

blood-Pb ranging from 4 to 40 µg/dL and that the number of cries was inversely related to cord blood-Pb. These data may parallel Pb-induced changes in vocalization seen in developing rats (see Section 5.3.5).

Earlier studies showed developmental lags in gross activity in rats with blood-Pb levels as low as 14 µg/dl, but other studies have found often contradictory results. More recent nonhuman primate studies showed either no effects or subtle motor impairments, increased durations of activity, failures to habituate, increased agitation, and fear. Rodent studies showed either no effects or increases in locomotor activity and changes in vocalization patterns. Thus, no clear pattern of Pb-induced effects on motor activity has yet emerged, though many studies do point to an increase in activity, as seen with epidemiologic findings. However, Cory-Slechta (1989), in discussing behavioral endpoints in Pb neurotoxicity, suggests that motor activity has little correspondence with more complex functions important for human populations.

8.4.2.5 Neurochemical Alterations

Examination of Pb-induced biochemical alterations of the nervous system has largely been limited to laboratory animal toxicologic studies. Although the linkage of neurochemical alterations in animal to human neurobehavioral function is somewhat speculative, these studies do provide some insight into possible neurochemical mediators of Pb neurotoxicity.

As summarized in Section 5.3.2, it has long been well known that Pb^{2+} acts as a Ca^{2+} mimetic. This affects neurotransmitter release in a dose-dependent fashion at glutamatergic, cholinergic, and dopaminergic synapses. Glutamate, acetylcholine and dopamine systems play very important roles in both cognitive function and brain development in both laboratory animals and in humans. Extensive research has focused on chronic Pb exposure effects on NMDA receptors. Much of the data point to an inhibition of NMDAR and changes in the ontogeny of receptor subunit expression, though full characterization of the effects on specific subunits is not available.

Considerable research has also focused on interactions of Pb^{2+} and Ca-dependent kinases and phosphodiesterases. Lead alters the activity of many of these enzymes, which results in changes in CREB, the transcription factor that controls expression of genes involved in learning, memory, and synaptic plasticity. Protein kinase C (PKC) is also a Pb target, though the Pb

effects on PKC in the intact animal have not been fully characterized. Thus, possible relationships of Pb effects on this pathway to human cognitive function effects are not yet clear.

8.4.2.6 Assessment of Dose-Response Relationships for Neurotoxic Effects of Lead Exposure

An important consideration in assessing potential public health impacts associated with Pb exposure is whether concentration-response relationships are linear across the full exposure range or, rather, shows nonlinearity. Also of interest is whether any thresholds can be discerned for various types of health effects associated with Pb exposure. The 1986 AQCD/Addendum and 1990 Supplement concluded that neurotoxic effects were related to blood Pb levels of 10 to 15 µg/dL and possibly lower. Since then, the U.S. Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have also lowered their definition of an elevated blood Pb concentration to 10 µg/dL (CDC, 1991; WHO, 1995). Average blood-Pb levels in U.S. children ages 1 to 5 years decreased from 15 µg/dL in 1976-1980 to ~3 µg/dL in 1991-1994 (CDC, 2000; Pirkle et al., 1998), allowing more recent studies to examine the effects of low level Pb exposure on the neurodevelopment of children (as discussed in Section 6.2.3).

Several recent epidemiologic studies have observed significant Pb-induced IQ decrements in children with peak blood Pb levels <10 µg/dL (e.g., Canfield et al., 2003a; Lanphear et al., 2005) and, in some cases, possibly below 5 µg/dL (Bellinger and Needleman, 2003; Téllez-Rojo et al., 2006). The most compelling evidence for effects below 10 µg/dL, as well as a nonlinear relationship between blood Pb levels and IQ, comes from the international pooled analysis of seven prospective cohort studies (n = 1,333) by Lanphear et al. (2005). The slope for Pb effects on IQ was steeper at lower blood-Pb levels, as indicated by the cubic spline function, the log-linear model, and the piece-wise linear model. The shape of the spline function indicated that the steepest declines in IQ were at blood-Pb concentrations <10 µg/dL. Based on stratified analyses using two cut points, a maximal blood-Pb of 7.5 and 10 µg/dL, the effect estimate for children with maximal blood-Pb levels <7.5 µg/dL was significantly greater than for those with a maximal blood-Pb ≥7.5 µg/dL. Thus, recent epidemiologic evidence is highly indicative of Pb-induced neurocognitive deficits in children at blood Pb levels below 10 µg/dL and, possibly, as low as 5 µg/dL.

In addition to IQ, significant associations were observed at low blood-Pb levels for other neurotoxicity endpoints. In the large NHANES III study, children aged 6 to 16 years with concurrent blood Pb <5 μg/dL exhibited significant Pb-related decrements in Arithmetic and Reading scores (Lanphear et al., 2000), but the possibility of earlier somewhat higher peaks in blood-Pb levels of the same children around 2.5 years of age cannot be ruled out. Inverse relationships between exposure to Pb and attentional behaviors and executive function were also observed in cohorts where >80% of the children had blood Pb levels <10 μg/dL (Canfield et al., 2003b; Stiles and Bellinger, 1993). Other studies have also found significant Pb-induced impairments of neuromotor function (Després et al., 2005) and hearing (Osman et al., 1999; Schwartz and Otto, 1987, 1991) in children with blood-Pb levels <10 μg/dL. Collectively, these studies most clearly indicate that Pb is associated with various neurodevelopmental endpoints in children at blood-Pb levels as low as 5 to 10 μg/dL. However, the shape of the concentration-response curve has not been as extensively examined in these studies; thus, there is still some question as to whether, for endpoints other than IQ, larger effects per incremental dose occur at blood Pb levels <10 μg/dL.

As stated in Section 5.3.7, there is little if any evidence from experimental animal studies that allow for any clear delineation at this time of a threshold for neurotoxic effects of Pb. Neurobehavioral changes have been reported in rodent studies at blood-Pb levels of ~10 μg/dL, whereas neurochemical and neurophysiological changes have been reported at blood Pb levels of ~15 μg/dL. However, these levels do not necessarily indicate a threshold for such effects but, rather, may only reflect the levels of exposure that have been studied to date. Also, other information, discussed in Chapter 4, suggests that blood-Pb concentrations in some animal models (e.g., the rat or other rodents) may be more comparable to somewhat lower blood-Pb levels seen in humans with more or less similar exposure conditions. Thus, blood-Pb levels associated with neurobehavioral effects appear to be reasonably parallel between humans and animals at reasonably comparable blood-Pb concentrations; and such effects appear likely to occur in humans ranging down at least to 5-10 μg/dL, or possibly lower (although the possibility of a threshold for such neurotoxic effects cannot be ruled out at lower blood-Pb concentrations).

Lead appears to exhibit a curvilinear, or U-shaped, dose-effect relationship for a number of toxicological endpoints. This effect is not unique to Pb, but occurs with other toxicants (e.g., mercury chloride, chlordane, toluene, chlorpyrifos) as well, as reviewed by Calabrese

(2005). In the case of Pb, this nonlinear dose-effect relationship occurs in the pattern of glutamate release (Section 5.3.2), in the capacity for long term potentiation (LTP; Section 5.3.3), and in conditioned operant responses (Section 5.3.5). The 1986 Lead AQCD also reported U-shaped dose-effect relationships for maze performance, discrimination learning, auditory evoked potential, and locomotor activity. Davis and Svendsgaard (1990) reviewed U-shaped dose-response curves and their implications for Pb risk assessment. An important implication is the uncertainty created in identification of thresholds and "no-observed-effect-levels" (NOELS). As a nonlinear relationship is observed between IQ and low blood Pb levels in humans, as well as in new toxicologic studies wherein neurotransmitter release and LTP show this same relationship, it is plausible that these nonlinear cognitive outcomes may be due, in part, to nonlinear mechanisms underlying these observed Pb neurotoxic effects.

8.4.2.7 Susceptibility and Vulnerability to Neurotoxic Effects from Lead Exposure

Several factors have emerged as likely affecting the relative likelihood that humans or laboratory animals may experience Pb-induced neurotoxic effects under particular Pb exposure conditions. Among the more important factors identified thus far are: age; gene-environment interactions; gender; and socioeconomic status.

Age

Identifying discrete periods of development when the fetus or child is particularly susceptible to Pb's effects on neurodevelopment is difficult as (1) age strongly predicts the period of peak exposure (around 18-27 months when there is maximum hand-to-mouth activity), making it difficult to distinguish whether greater neurotoxic effects resulted from increased exposure or enhanced susceptibility at a particular age; and (2) despite changes in actual blood Pb levels, children tend to maintain their relative rank order with regard to neurodevelopment indicators through time, limiting the ability to examine critical periods of development.

One notable epidemiologic study has observed the strongest associations between IQ at school age and academic achievement and blood Pb concentrations at 2 years of age (Bellinger, et al., 1992). An understanding of human neurodevelopmental biology supports the notion that the first 3 years of life represent a particularly vulnerable period. Maximal ingestion of Pb often coincides with this same period of time when major events are occurring in the development of

the human central nervous system, including some neurogenesis, rapid dendritic and axonal outgrowth, synaptogenesis, synaptic pruning, and programmed cell death (see Nolte, 1993).

However, the human central nervous system continues to mature and be vulnerable to neurotoxicants throughout the lifespan (Selevan et al., 2000, Weiss, 2000, Rice and Barone, 2000). Several prospective studies of children with both high and low Pb exposures found concurrent or lifetime average blood-Pb levels to be more strongly associated than other earlier blood-Pb measures with school age IQ and other measures of neurodevelopment (Canfield et al., 2003a; Dietrich et al., 1993a,b; Tong et al., 1996; Wasserman et al., 2000b). Using data from the Treatment of Lead-Exposed Children (TLC) study, Chen et al. (2005) examined whether cross-sectional associations observed in school age children 84-90 months of age represented residual effects from 2 years of age or "new" effects emerging among these children. Concurrent blood-Pb concentration always had the strongest association with IQ. The strength of the cross-sectional associations increased over time, despite lower blood-Pb concentrations in older children. Adjustment for prior IQ did not fundamentally change the strength of the association with concurrent blood-Pb level. These results suggest that Pb exposure continues to be toxic to children as they reach school age, but does not support an interpretation that all of the damage occurred by the time the child reaches 2 to 3 years of age. Examination of the toxicologic evidence may be especially enlightening on this topic, given the difficulties involved in assessing any periods of particularly increased susceptibility to Pb neurodevelopmental health effects in the epidemiologic setting.

Cory-Slechta (1989) has reviewed age considerations in the neurotoxicology of Pb and concluded that: (1) though the presumed critical exposure period is prenatal and neonatal, vulnerability extends well beyond this period in both rodents and humans; (2) for some neurobehavioral endpoints (such as schedule-controlled behavior), the developmental period of exposure can be relatively unimportant, whereas the body burden of Pb is more critical; (3) enhanced vulnerability to Pb may also occur in later life, as ageing processes induce degenerative changes in various organ systems; and (4) age-related shifts occur in the toxicokinetics of Pb, such that Pb can be redistributed to brain and liver from bone during later phases of life beyond the time of earlier Pb exposures.

Gene-Environment Interactions

A few recent epidemiologic studies have examined susceptibility to Pb health effects as related to genetic polymorphisms associated with Pb biokinetics and/or neurotransmitter metabolism and function (as discussed in Section 6.2.10). Genetic polymorphisms in certain genes have been implicated as influencing the absorption, retention and toxicokinetics of Pb in humans. Although the ALAD gene has been the most studied, as of yet, the consequences of different alleles for susceptibility to the neurodevelopmental consequences of Pb exposure in children are unclear. For example, ALAD2 polymorphism has been implicated in influencing vulnerability by raising blood Pb levels or by decreasing them by maintaining Pb in a sequestered state in the bloodstream. Suggestive but limited evidence appears to indicate that adolescents with the ALAD2 polymorphism tended to have lower dentin-Pb levels and performed better in areas of attention and executive functioning when compared to subjects with the ALAD1 polymorphism.

Another gene of interest is the vitamin D receptor or VDR gene, which is involved in calcium absorption through the gut. The variant VDR alleles may modify Pb concentrations in bone and the rate of resorption and excretion of Pb over time. The relationship between the VDR Fok1 polymorphism and blood Pb concentrations was evaluated in 275 children enrolled in the Rochester Longitudinal Study. A significant interaction was found between floor dust-Pb loading and VDR-Fok1 genotypes on blood Pb concentration, with the FF genotypes (a marker for increased calcium absorption) having the highest adjusted mean blood Pb concentrations at 2 years of age compared to children with Ff or ff genotypes. High prevalence of FF genotypes in African-American children, compared to non-African American children, may partially explain higher blood Pb concentrations often observed in African-American children. There have been no studies to indicate which, if any, of the VDR polymorphisms are associated with increased vulnerability to the neurodevelopmental toxicity of Pb. Animal toxicology studies have yet to identify any role of genetic polymorphism in ALAD or VDR in affecting Pb toxicity.

Tiffany-Castiglioni et al. (2005), in an overview of genetic polymorphisms relating to mechanisms of neurotoxicity, state that an understanding of the relationship among ALAD polymorphisms, blood Pb levels, and Pb neurotoxicity is difficult at this time. They further note that, though urinary ALA is a good marker for Pb exposure, it may not correlate with neuronal

damage. There is a similar lack of information using animal models to characterize genetic polymorphisms of the VDR and hemochromatosis genes.

Gender

Most surveys find that boys have higher blood-Pb levels than girls; yet the data are less clear with regard to gender-related differences in Pb-associated neurodevelopmental effects. As discussed in Section 6.2.10, a greater male vulnerability was seen in the Cincinnati Lead Study at various assessments from birth to adolescence. Also, data from a cross-sectional study in England showed more pronounced Pb-IQ deficit associations for boys at 6 years of age. However, in a study of 764 children in Taiwan, the relationship between Pb exposure and IQ scores was much stronger in girls; and, in the Port Pirie, Australia cohort study, Pb effects on cognition were significantly stronger in girls at ages 2, 4, 7, and 11-13 years.

In the Cincinnati Lead Study (see Section 6.2.3.1), an extensive neuropsychological battery administered to 15-17 year old subjects examined executive functions, attention, memory, achievement, verbal abilities, visuoconstructional skills, and fine-motor coordination as key endpoints. About 30% of the subjects had blood-Pb concentrations ≥25 µg/dL during the first 5 years of life, and 80% of the cohort had at least one blood Pb ≥15 µg/dL. A strong "executive functions" factor did not emerge from a factor analysis of scores. However, the analysis, following covariate-adjustment, revealed strong associations between Pb exposure and the attention factor for males. This gender interaction suggests that neuromechanisms sub-serving attention were affected by Pb in this cohort for boys but not for girls. This is not surprising, given the heightened vulnerability of males for a wide range of developmental perturbations. A substantial gender difference (greater incidence among boys) in the incidence of Attention Deficit/Hyperactivity Disorder (ADHD) is well established, and one could speculate that early exposure to Pb exacerbates a latent potential for such problems.

The Port Pirie, Australia cohort study examined relationships between Pb exposure and emotional and behavioral problems at age's 11-13 years after stratifying the data set by gender. Stronger associations with Pb were observed for externalizing behavior problems in boys versus girls; but greater internalizing behavior problems were found for girls.

Early laboratory animal Pb toxicology studies did not evaluate gender differences in responses to chronic or acute Pb exposure, with the exception of several that showed differences

in social investigatory behavior and nonsocial activity. Some studies pointed to greater social investigatory behavior in males compared to females. More recent work by Cory-Slechta and colleagues (Section 5.3.1.7) has shown greater synergistic effects of Pb and stress in female rats, coupled with permanently elevated corticosterone levels. Also, maternal Pb exposure and restraint stress caused greater changes in operant behavior and stress responses in female offspring. These studies point to clear gender differences in response to Pb and suggest possible hypothalamic-pituitary-adrenal axis-modulated effects of Pb on CNS function.

Socioeconomic Status

Epidemiologic studies have shown that Pb exposure is typically higher among low socioeconomic status (SES) children compared to other U.S. children. Chronic stress and consequent increased levels of glucocorticoids are also associated with low SES. Cory-Slechta et al. (2004) have pointed out that both elevated glucocorticoids and Pb can cause similar behavioral changes and that both impact the mesocorticolimbic systems of the brain. As discussed in Section 5.3.7, their data indicate a potential mechanism whereby Pb exposure enhances susceptibility to cognitive deficits and disease states.

8.4.2.8 Persistence/Reversibility of Neurotoxic Effects from Lead Exposure

Much of the classic Pb poisoning literature substantiates well the persistence of serious neurological damage resulting from extremely high Pb exposures. The persistence of more subtle, but important, neurotoxic effects of lower level Pb exposure has been accorded much attention during the past decade or so. Much of the pertinent human and animal data seem to suggest that the neurotoxic effects of Pb may not generally be reversible. As noted in Chapter 6, excessive accumulation of Pb in childhood has latent and/or persistent adverse health effects on both the peripheral and central nervous systems of human adults assessed 19-29 years later. Also, chelation studies in humans and animals (summarized in Tables AX6-2.10 and AX5-3.6) show that chelation decreases total body Pb burden, but does not necessarily exert evident effects on Pb-induced cognitive deficits. For example, the extensive multi-center TLC study summarized by Rogan et al. (2001) indicates that medical interventions involving chelation therapy (e.g., Succimer use) do not seem to fully reverse cognitive deficits associated with early Pb exposure. Also, nonhuman primate studies evaluated the persistence of effects by limiting the

Pb exposures to the first year of life, as discussed in the 1986 AQCD and more recently (see Section 5.3.5). In these monkeys, deficits in performance of both spatial discrimination tasks and delayed spatial alternation were seen up to 8 years post exposure, when blood Pb had dropped to control levels. In one study, however, the Pb-treated monkeys performed better than control subjects at 4 years of age. In addition, a few studies discussed in the 1986 Lead AQCD and some more recent studies have suggested possible reversibility of observed Pb-induced learning deficits. Such studies suggest that reversibility depends on the age of the organism at the time of exposure, the exposure duration, dosage, and other exposure parameters. Also, several animal studies (Section 5.3.5) demonstrate that environmental enrichment during development may, at times, help to engender some recovery from cognitive effects of earlier short-term low-level Pb exposures.

Davis et al. (1990), however, sound a cautionary note with regard to the interpretation of neurobehavioral data in light of compensatory capacities of the nervous system. They note that compensatory capacities may become overwhelmed with aging, concurrent disease state, stress due to socioeconomic status, or other stressors. It may be only then, possibly decades following earlier Pb exposure, that some Pb-induced neurobehavioral effects are manifested.

8.4.2.9 Summary of Toxicologic and Epidemiologic Evidence of Lead-Induced Neurotoxicity

Findings from numerous experimental studies of rats and of nonhuman primates, as discussed in Chapter 5, parallel the observed human neurocognitive deficits and the processes responsible for them. Learning and other higher order cognitive processes show the greatest similarities in Pb-induced deficits between humans and experimental animals. Deficits in cognition are due to the combined and overlapping effects of Pb-induced perseveration, inability to inhibit responding, inability to adapt to changing behavioral requirements, aversion to delays, and distractibility. Higher level neurocognitive functions are affected in both animals and humans at very low exposure levels ($\leq$10 µg/dL), more so than simple cognitive functions. For example, the discrimination reversal paradigm is a more sensitive indicator of Pb-induced learning impairment than simple discrimination. Many studies suggest that most Pb-induced cognitive deficits are very persistent and that animals remain vulnerable to the effect of Pb throughout development. Some studies, however, suggest that environmental enrichment during

early development may confer some offsetting protection against Pb-induced cognitive effects or that other factors (e.g., short-lived exposure duration/low concentration) may, at times, induce detectable but transient cognitive deficits. Also, more evidence is emerging that substantiates Pb-induced attentional deficits, which may contribute to persisting cognitive dysfunction, poorer academic performance, and/or maladaptive anti-social behavior patterns (e.g., delinquency).

Other behavioral endpoints (e.g., social behavior, aggression, and locomotor activity) evaluated in animal studies in relation to Pb exposure did not clearly indicate Pb-induced impairments. This may be due to the lack of effect with low-level Pb exposure or to variables (e.g., nutrition, age, gender, and strain) possibly not well controlled for experimentally.

8.4.3 Cardiovascular Effects

Epidemiologic studies that have examined the effects of blood-Pb levels on blood pressure have generally found positive associations, even after controlling for confounding factors such as tobacco smoking, exercise, body weight, alcohol consumption, and socioeconomic status (discussed in Section 6.5.2). Recent meta-analyses of these studies have reported robust, statistically-significant, though small effect-size, associations between blood-Pb concentrations and blood pressure. For example, the meta-analysis of Nawrot et al. (2002) indicated that a doubling of blood Pb corresponded to a 1 mm Hg increase in systolic blood pressure. Although this magnitude of increase is not clinically meaningful for an individual, a population shift of 1 mm Hg is important. The majority of the more recent studies employing bone-Pb level have also found a strong association between long-term Pb exposure and arterial pressure. Since the residence time of Pb in blood is relatively short but very long in bone, the latter observations have provided compelling evidence for the positive relationship between Pb exposure and a subsequent rise in arterial pressure in human adults.

Numerous experimental animal studies have shown that exposure to low levels of Pb for extended periods results in an eventual onset of arterial hypertension (HTN) that persists long after the cessation of Pb exposure in genetically normal animals. Many studies have been conducted to explore the mechanisms by which chronic Pb exposure may cause HTN. Most of these studies have examined various blood-pressure regulatory and vasoactive systems in animal models of Pb-induced HTN. A number of studies have also utilized in vitro cell culture systems such as endothelial and vascular smooth muscle cells to gain insight into molecular mechanisms

implicated in this process. Key findings have emerged from the newly available in vivo and in vitro studies of mechanisms including the following several important points.

During the past decade, several studies have shown that Pb exposure causes oxidative stress, particularly in the kidney and cardiovascular tissues, as well as in cultured endothelial and vascular smooth muscle cells (VSMC), as noted in Section 5.5.2.1. The in vivo studies have further shown that Pb-induced oxidative stress is, at least in part, responsible for associated hypertension (HTN) in experimental animals. Khalil-Manesh et al. (1994) were among the first to suggest that oxidative stress may be involved in the pathogenesis of lead-induced HTN. Gonick et al. (1997) later provided evidence for the occurrence of oxidative stress and compensatory up regulation of NOS isotypes in the kidney of animals with lead-induced HTN. Studies carried out with antioxidants, e.g., lazaroid compound, resulted in a significant alleviation of oxidative stress, improved NO availability, and a marked attenuation of HTN without affecting blood Pb concentration, further demonstrating that Pb-induced HTN is associated with diminished NO availability and that the latter was mediated by oxidative stress (Vaziri et al., 1997).

Numerous in vivo and in vitro studies on Pb-induced HTN, using endothelial and VSMC with or without intervention by antioxidant therapeutics, suggest a role of oxidative stress and NO in the pathogenesis of lead-induced HTN in the rat.

These observations provided compelling evidence that Pb-induced HTN causes oxidative stress, which, in turn, promotes functional NO deficiency via ROS-mediated NO inactivation. The latter, in turn, participates in the development and maintenance of HTN and cardiovascular abnormalities. Also, the formation of the highly cytotoxic reactive nitrogen species peroxynitrite ($ONOO^-$) from the NO-ROS interaction and the associated nitrosative stress could potentially contribute to long-term cardiovascular, renal, and neurological consequences of Pb exposure.

Higher plasma levels of lipid peroxides in uncontrolled essential hypertension as compared to normal controls suggested that free radicals are involved in the pathobiology of human essential hypertension. Angiotensin II, a potent vasoconstrictor, was found to stimulate free radical generation in normal leukocytes. This increase in free radical generation was thought to inactivate NO, and possibly prostacyclin, which can lead to an increase in peripheral vascular resistance and hypertension.

In spite of such a wide range of experimental investigations into the cardiovascular effects of Pb in animal studies, it is still not clear as to why low, but not high, levels of Pb exposure cause HTN in experimental animals.

8.4.4 Heme Synthesis and Blood Effects

Lead exposure has long been recognized to be associated with disruption of heme synthesis in both human children and adults. With extreme Pb exposure leading to blood-Pb levels >30 μg/dL, Pb-induced heme synthesis interference leads to notable reductions in hemoglobin synthesis and, at blood-Pb ≥40 μg/dL, to frank anemia (a classic clinical sign of severe Pb poisoning).

Other indications of disruption of heme synthesis are readily detectable at distinctly lower blood-Pb concentrations, but mainly tend to serve as highly useful biomarkers of Pb exposure. Elevated blood-Pb concentrations of ~20–30 μg/dL, for example, are sufficient to halve erythrocyte ALAD activity and sufficiently inhibit ferrochelatase so as to double erythrocyte protoporphyrin (EP) levels. Erythrocyte ALAD activity ratio (the ratio of activated/non-activated enzyme activity) has been shown to be a sensitive, dose-responsive measure of Pb exposure, regardless of the mode of Pb administration. Competitive enzyme kinetic analyses in RBCs from both humans and cynomolgus monkeys indicated similar inhibition profiles by Pb. Decreased ALAD activity in rat RBCs have been reported at blood Pb levels of 10 μg/dL.

The effects of various metals, including Pb, on RBC porphobilinogen synthase (PBG-S) have been studied using human RBC hemolysate (see Section 5.2.3). Effects on the enzyme were found to depend on the affinity of the metal for thiol groups at its active sites. Additional studies utilizing rabbit erythrocyte PBG-S indicate that Pb acts as a potent effector of this enzyme both in vitro and in vivo. Increased erythrocyte protoporphyrin levels seen at blood-Pb concentrations ≥15 μg/dL represent another widely used biomarker of Pb exposure.

Comparison of pyrimidine-5′-nucleotidase (P5N) and deoxypyramidine-5-nucleotidase levels in the RBC of Pb-exposed workers and matched controls also showed significantly lower levels of P5N in Pb-exposed workers. Similar observations were reported for neonatal rat RBCs, with the low levels of nucleotides being hypothesized to be due to inhibition of P5N activity by Pb, as the depression in enzyme activity was correlated with blood-Pb levels (see Section 5.2.5).

8.4.5 Renal System Effects

The nephrotoxic effects of Pb are mediated by alterations in the glomerular filtration rate (GFR). A battery of tests used to screen both environmentally- and occupationally-exposed individuals often include: (1) measures of glomerular integrity, (2) tubular absorption and secretion, (3) measure of tubular integrity, (4) measure of glomerular and distal tubular function, (5) glomerular structural proteins, and (6) measure of distal tubular function. Numerous new epidemiologic studies discussed in Chapter 6 provide important new findings on associations between Pb exposure and impacts on renal function (see Section 6.4).

Of particular importance are new analyses of associations between blood-Pb and renal outcomes in 15,211 adult subjects in the NHANES III study (conducted during 1988-1994). Dichotomous renal outcome measures analyzed included elevated serum creatinine and chronic kidney disease. Mean blood-Pb level was 4.2 μg/dL among the 4,813 hypertensives and 3.3 μg/dL in normotensives, with prevalence of elevated serum creatinine being higher among hypertensives than nonhypertensives but prevalence of chronic kidney disease being similar. The authors noted that (1) the associations were strong, dose-dependent and consistent before and after adjustment (e.g., for age, race, and gender) and (2) higher blood Pb was associated in nonhypertensives with higher prevalence of chronic kidney disease in diabetics. This study is notable for sample size, comprehensive adjustment for other renal risk factors, and the fact that the study population is representative of the general U.S. population. In another study of 820 women (ages 53-64 years) in Sweden, significant negative associations were seen between blood Pb (mean blood Pb of 2.2 μg/dL) and both glomerular filtration rate (GFR) and creatinine clearance, an association that was apparent over the entire dose range. This study also had the additional advantage of blood and urinary cadmium assessment.

The above studies and other general population studies constitute some of the most important types of research on Pb renal effects during the past 20 years, as discussed in Section 6.4.4.1. Overall, a number of strengths are present in this body of literature. These include study design with longitudinal data in some studies; large populations in both the United States and Europe; comprehensive assessment of Pb dose (including use of bone Pb as a measure of cumulative Pb body burden in some studies); and statistical approaches using a range of exposure and outcome measures, while adjusting for numerous renal risk factors. Associations

between Pb exposure and worse renal function were observed in most of the general population studies.

Residual confounding and reverse causality have both been proposed as alternative explanations for the reported associations between Pb and renal dysfunction. As discussed in Section 6.5, increased blood pressure has been associated with Pb exposure in general populations. Adjustment for hypertension or blood pressure, although typical in Pb-renal studies, carries the risk of underestimating the actual slope of the association between Pb dose and renal dysfunction. Given the careful adjustment for confounding in the Pb-renal general population literature, it is thought that residual confounding is not a likely explanation for the observed Pb-renal dysfunction associations. Reverse causality, i.e. attributing increased Pb dose to reduced Pb excretion as a consequence of renal insufficiency, is another possible explanation posed to explain such associations. However, by examining temporal relationships between Pb dose and renal function in longitudinal studies, it has been convincingly shown that Pb dose predicts decline in renal function. Other evidence against possible reverse causality is the positive impact of Pb chelation on renal function (see Section 6.4.4.3), although the possibility of a direct beneficial effect of chelating agents on renal function cannot be ruled out.

Increased risk for nephrotoxicity has been observed at the lowest Pb exposure levels studied epidemiologically to date. More specifically, the newly available general population studies have shown associations between blood Pb and indicators of renal function impairment at blood-Pb levels extending below 10 μg/dL, with nephrotic effects having been reported among some adults with mean concurrent blood-Pb levels as low as ~2 to 4 μg/dL. However, the data available to date are not sufficient to determine whether nephrotoxicity is related more to such current blood-Pb levels, higher levels from past exposures, or both. An association between cumulative Pb dose (indexed by mean tibia Pb of 21.5 μg/g bone mineral) and longitudinal decline in renal function has been observed as well. Blood Pb levels <10 μg/dL have also been associated with altered creatinine clearance, as noted in Chapter 6 (Figure 6-8). Slopes ranged from 0.2 to −1.8 mL/min change in creatinine clearance per each μg/dL increase in blood Pb.

Animal toxicology studies reported that both low and high dose Pb-treated animals showed a "hyperfiltration" phenomenon during the first 3 months of Pb exposure. This finding could be invoked as a partial explanation for late changes of glomerulosclerosis seen in high-Pb dose animals but cannot explain the lack of glomerular changes in the low-dose animals. These

results support observations by several investigators in humans, leading some to argue that Pb nephropathy should be added to diabetic nephropathy as diseases that lead to early hyperfiltration. Also, animal toxicology studies that evaluated biochemical alterations in Pb-induced renal toxicity suggest a role for oxidative stress and involvement of NO, with a significant increase in nitrotyrosine and substantial fall in urinary excretion of NO_x.

A few animal toxicology studies that evaluated the effect of coexposure to other metals indicated that cadmium increases Pb in blood when both are given, but diminishes Pb in liver and kidney. Selenium, an antioxidant, improves both parameters, as does thiamine or L-lysine plus zinc. Iron deficiency increases intestinal absorption of Pb and the Pb content of soft tissues and bone. Aluminum decreases kidney Pb content and serum creatinine in Pb-intoxicated animals. Age also has an effect on Pb retention. There is higher Pb retention at a very young age but lower bone and kidney Pb at old age, attributed in part to increased bone resorption and decreased bone accretion and kidney Pb.

The above findings appear to indicate likely associations between some indicators of altered kidney function (e.g., increased creatinine clearance) at relatively low blood-Pb levels among the general population. However, the potential public health significance of such findings is difficult to discern just yet. This is especially true in light of difficulties in resolving discrepancies between these newly reported findings in the general population studies versus observation among the more than 10,000 occupationally-exposed workers studied of notable Pb effects on renal tubular function only when blood-Pb exceeds distinctly higher levels (e.g., >30-40 μg/dL).

8.4.6 Lead-Associated Immune Outcomes

The effects of Pb exposure on the immune system of animals are described in Section 5.9 and are summarized in Figure 5-18. These include the targeting of T cells and macrophages by Pb. Lead-induced alterations center on an increased inflammatory profile for macrophages (i.e. elevated tumor necrosis factor-alpha, oxygen radical, and prostaglandin production) and a skewing of the T cell response away from T helper 1 (Th1)-dependent functions toward T helper 2 (Th2)-dependent functions. The resulting immune changes include an increased production of Th2 cytokines (e.g., IL-4, IL-10) and certain immunoglobulins [e.g., immunoglobulin E (IgE)]. Concomitantly, there is a decrease in Th1-associated cytokines

(interferon gamma and IL-12) and in Th1-functions, e.g., the delayed type hypersensitivity (DTH) response. Significant age-related differences in immunotoxic sensitivity to Pb (based on blood-Pb concentrations) approximate an order of magnitude difference in sensitivity between the perinatal period and adulthood (see Table 5-10). Importantly, immune changes are associated with blood-Pb levels well below 10 µg/dL following gestational or perinatal exposures (see Table 5-9). However, major immune cellular alterations are not a hallmark of low-level Pb exposure, despite significant Pb-induced shifts in immune function. This lack of major immune cell population changes becomes important for interpretation of human epidemiologic results.

Human epidemiologic immune evaluations are hampered by the reality that the most informative sources of functionally-reactive immune cells (e.g., those responding to antigens in the lymphoid organs and local lymph nodes) are not available for routine human sampling. This can be important in considerations of early-life associated immunotoxicity where functional assessment of immune changes appear to be particularly important, as noted by Dietert and Piepenbrink (2006). Instead, circulating lymphocytes and serum or plasma immunoglobulin levels in humans must serve as easily accessible surrogates for a more comprehensive determination of immune status. Despite this inherent limitation, the animal and human data for Pb-induced immune alterations are in general agreement, including the association of blood-Pb levels below 10 µg/dL with significant neonatal/juvenile immune alterations.

The sentinel result suggesting that low-level Pb exposure produces similar immune changes among animals and humans is the positive association of blood-Pb levels with IgE level. This association has been observed at blood-Pb levels <10 µg/dL following early life exposure in both humans (Section 5.9.3.2) and animals (Section 5.9.3). Other animal studies also support this by showing low-level Pb-induced increases in neonatal/juvenile IL-4, the hallmark cytokine modulating IgE production. Similarly, in the adult human, a positive association between blood-Pb level and IgE level has been reported for occupationally-exposed workers. It is not surprising that human epidemiologic results showed less consistent changes in other immunoglobulins, since Th biasing would be expected to produce shifts among immunoglobulin G (IgG) subclasses without necessarily changing overall IgG concentrations, consistent with results in the rat.

It should be noted that, prior to 1992, no human epidemiology study involving Pb reported comparisons of IgE levels. Also, since IgE is a minor immunglobulin component of

human serum, several studies since 1992 did not include IgE quantitation in the evaluation. The animal data suggest that IgE (as well as the supporting cytokine, IL-4) are among the most sensitive parameters for modulation following low-level Pb exposure. Thus, in retrospect, those human studies that did not evaluate IgE levels may have focused on Pb-insensitive immune parameters.

Cell-mediated immunity in animals evaluated by the Th-dependent DTH reaction in most animal studies (see Section 5.9.4) showed that this measure was particularly sensitive to Pb-induced immunosuppression. In humans, the primary surrogate for cell-mediated immunity was a non-functional measure of circulating leukocyte populations. Despite this difficulty in evaluation, a majority of studies (see Table 6-6) that quantitated T, Th, Tc, and B cells reported decreases in either T or Th cells relative to an increase in circulating B cells. This is consistent with the profile described in the animal studies, where Th promotion of cell mediated immune function is impaired by Pb exposure while humoral immunity remained either unchanged or displayed increased IgE production.

Numerous animal studies reported that Pb produced elevated levels of TNF-alpha, superoxide anion and prostaglandins while depressing production of nitric oxide by macrophages (see Section 5.9.6). To the extent the same endpoints have been examined, the results are similar between animals and humans. One study has reported that in vitro-activated monocytes from Pb-exposed children were depressed in nitric oxide production, contrasting with a positive association between blood-Pb level and production of superoxide anion. Based on these results, the pattern of Pb-induced changes in major macrophage metabolites appears to be similar between the animal experimental and human epidemiological data.

Comparison of the human and animal studies is quite feasible and is limited only by the number of studies that incorporated comparable immune endpoints. In retrospect, several prior human epidemiological studies measured endpoints that appear to be Pb-insensitive based on the most recent animal data. Of the studies that evaluated similar parameters, the results are strikingly in agreement.

8.4.7 Reproduction and Development Effects

The majority of the experimental animal studies of Pb effects on reproduction and development examined effects due to inorganic forms of Pb, with very little being known about reproductive and developmental effects of organic Pb compounds.

Timing of exposure has been found to be critical to Pb-induced male reproductive toxicity in rats. Studies conducted in nonhuman primates support the importance of exposure timing and indicate that the adverse effects of Pb on male reproduction are dependent upon age (i.e., developmental stage at time of exposure) and duration of exposure. Numerous more recent studies conducted in experimental animals support the earlier findings that Pb exposure during early development can delay the onset of male puberty and alter reproductive function later in life (see Section 5.4.2.1).

Other recent research supports the conclusion that mechanisms for endocrine disruption in males involve Pb acting at multiple sites along the hypothalamic-pituitary-gonadal (HPG) axis. However, variable findings regarding specific types of Pb effects have been attributed to complex mechanisms involved in hormone regulation and the multiple sites of action for Pb. It has been suggested that differences in results among studies may, in part, be attributed to an adaptive mechanism in the hypothalamic-pituitary-gonadal axis that may render the expression of some toxic effects dependent on dose and exposure duration (see Section 5.4.2). Thus, adaptive or multiple effects on the HPG axis having different dose-duration-response relationships may explain apparent inconsistencies among reported Pb effects on circulating testosterone levels, sperm count, and sperm production.

A possible mode of action for Pb-induced testicular injury is oxidative stress (as discussed in Section 5.4.2.4). Pb-induced oxygen free radical generation has been suggested as a plausible mechanism of testicular injury in primates. This oxygen radical hypothesis is supported by studies conducted in rodents; and the oxidative stress hypothesis is supported by observations of increases in the percentage of apoptotic cells in the testes of rodents in response to Pb exposure.

Several modes of action for Pb-induced, endocrine disruption-mediated, alterations in female reproduction have also been proposed, as discussed in Section 5.4.3. These include changes in hormone synthesis or metabolism at the enzyme level and changes in hormone receptor levels. In addition, Pb may alter sex hormone release and imprinting during early

development. The latter effects would be consistent with observations of persistent changes in estrogen receptor levels in the uterus and altered ovarian LH function in Pb-exposed animals.

A persistent effect of maternal Pb exposure (blood Pb 30 to 40 µg/dL) has been seen on corticosteroid levels in adult offspring (as discussed in Section 5.4.6). Both male and female offspring born to dams exposed to Pb exhibited elevated corticosteroid levels as adults. In female offspring, the Pb effect was potentiated when maternal Pb exposure occurred in combination with environmental stress (administered as restraint). The interplay between Pb and stress hormones is consistent with other animal toxicologic findings wherein neonatal exposure to Pb (blood Pb 70 µg/dL) decreased cold-water swimming endurance (a standard test for stress endurance).

The literature provides convincing support for Pb-induced impairment of postnatal growth, as discussed in Section 5.4.5. Although some early studies ascribed the reduction in postnatal growth to reduced food consumption (suggesting an effect of Pb on satiety mechanisms), more recent studies report impaired growth unrelated to changes in food consumption. These and other findings suggest that Pb exposure may impair growth through a mechanism that involves a suppressed pituitary response to hypothalamic stimulation. The mechanism may involve Pb-induced reduction in plasma concentrations of IGF_1.

8.4.8 Bone and Teeth Effects

Lead is readily taken up and stored in the bone of experimental animals, where it can potentially manifest toxic effects that result in stunted skeletal growth. In experiments reported since the 1986 Lead AQCD (see Section 5.8.3), uptake and retention of Pb were determined in bone from rats exposed to plain water or water containing Pb-acetate (41.7 to 166.6 mg/L) for 12 to 16 weeks. After 4 weeks, the skeletal Pb in animals receiving the lowest dose was almost 5 times higher than in control animals (5.9 versus 1.2 µg Pb/g bone, respectively). Lead levels in bones from animals receiving 83.3 mg/L and 166.6 mg/L were dose-dependently higher (at 11.7 and 17.0 µg Pb/g bone, respectively) after 4 weeks of exposure.

Results from several new animal studies have also yielded evidence for Pb exposure adversely affecting bone growth and density, which may be potentially manifested through Pb interference with growth and hormonal factors as well as toxic effects directly on bone. One of

the studies suggested Pb was mediating its effect through 1, 25-$(OH)_2D_3$, rather than via a direct action on the Calbindin-D protein.

The fact that Pb exposure has been associated with altered bone metabolism and decreased growth and skeletal development is suggestive of potential Pb perturbation of one or more endocrine factors, e.g., growth hormone. However, overall, available rat studies suggest that differences in growth seen with Pb exposure may not necessarily be due to alterations in secretion of growth hormone. Rather, effects on calcium uptake and/or metabolism may be more crucial, as suggested by the results of several in vitro studies. The results suggest that the calcium-ATPases of intracellular stores are potentially poisoned by Pb entering the cells.

An invaluable method to explore the kinetics of Pb transfer from bone to blood has been developed and evaluated within the last decade (see Section 5.8.6). The method uses recent administration of sequential doses of Pb mixes enriched in stable isotopes (^{204}Pb, ^{206}Pb, and ^{207}Pb) to female cynomolgus monkeys that were earlier chronically administered a common Pb isotope mix (1,300 to 1,500 µg Pb/kg body weight/day for ≥10 years). The stable isotope mixes serve as a marker of recent exogenous Pb exposure, whereas the chronically administered common Pb serves as a marker of endogenous (principally bone) Pb. It was found that administration of the first isotope label allows measurement of the contribution of historic bone stores to blood Pb. Exposure to subsequent isotopic labels allows measurement of contributions from historic bone Pb stores and the recently administered enriched isotopes that incorporated into bone. In general, the contribution from historic bone Pb (common Pb) to blood Pb level was relatively constant (~20%), but was augmented by spikes in total blood Pb due to current administration of the stable isotopes (blood Pb ranged from 31.2 to 62.3 µg/100 g).

Using the above sequential stable isotope administration method, another study examined flux of Pb from maternal bone during pregnancy of 5 female cynomolgus monkeys previously exposed to common Pb (~1,100 to1,300 µg Pb/kg body weight) for about 14 years. In general, Pb levels in maternal blood (as high as 65 µg/100 g) attributable to Pb from mobilized bone dropped 29 to 56% below prepregnancy baseline levels during the first trimester of pregnancy. This was ascribed to the known increase in maternal fluid volume, specific organ enlargement (e.g., mammary glands, uterus, placenta), and increased metabolic activity that occurs during pregnancy. During the two later trimesters, when there is a rapid growth in the fetal skeleton and compensatory demand for calcium from the maternal blood, the Pb levels increased up to 44%

over pre-pregnancy levels. Blood-Pb levels in the fetus generally corresponded to those found in the mothers, both in total Pb concentration and in the proportion of Pb attributable to each isotopic signature dose. From 7 to 25% of the Pb found in fetal bone originated from maternal bone, with the balance derived from oral dosing of the mothers with isotope during pregnancy. Of interest, in offspring from a low Pb exposure control monkey (blood Pb <5 µg/100 g) ~39% of Pb found in fetal bone was of maternal origin, suggesting enhanced transfer and retention of Pb under low Pb conditions.

These studies show that Pb stored in bone is mobilized during pregnancy and lactation, exposing both mother and fetus/nursing infant to potentially toxic blood/milk Pb levels. Also of much concern, a significant proportion of Pb transferred from the mother is incorporated into the offspring's developing skeletal system, where it can serve as a continuing source of toxic exposure. The latter study illustrates the utility of sequentially administered stable isotopes in pregnancy; however, its use may also be applicable in studies of lactation, menopause, osteoporosis, and other disease states where mobilization of bone and release of Pb stores occurs. Further, given that isotopic ratios of common Pbs vary by location and source of exposure, when humans migrate from one area and source of exposure to another, it is possible to document changes in mobilized Pb, especially during times of metabolic stress.

During pregnancy, transfer of Pb from mother to offspring has been documented. Still, other available evidence also suggests that a more significant transfer from mother to offspring occurs during lactation, when the Pb concentration in mother's milk can be several times higher than corresponding blood-Pb levels.

8.4.9 Hepatic and Gastrointestinal System Effects

A large body of experimental animal toxicology database reviewed in this document indicated hepatotoxic effects, including liver hyperplasia, at very high dose Pb exposures. Based on the limited data available in these toxicology studies on blood-Pb levels, inhibition of liver heme synthesis and inhibition of liver ALAD was reported at 15-20 µg/dL, whereas alterations in liver cholesterol metabolism and induction of hepatic oxidative stress was observed at 20-30 µg/dL.

Studies of hepatic enzyme levels in serum suggest that liver injury may be present in lead workers; however, associations specifically with Pb exposures were not evident. Also, children

exposed to relatively high Pb levels (blood-Pb >30 μg/dL) exhibit depressed levels of circulating 1,25-dihydroxy vitamin D (1,25-$(OH)_2D_3$). However, associations between serum vitamin D status and blood Pb were not evident in a study of calcium-replete children who had average lifetime blood-Pb concentrations <25 μg/dL.

Investigations into the potential molecular mechanisms involved in these alterations suggest induction of gene expression for CYP51 (Lanosterol 14α-demethylase), an essential enzyme for cholesterol biosynthesis, in Pb-nitrate-induced liver hyperplasia, although other cytochrome P450 enzymes involved in drug metabolism have been reported as being suppressed. The induction of the cytokines interleukin-1α and TNF-α in rat liver prior to the induction of the genes for these synthesis enzymes suggested that Pb-nitrate-induced cholesterol synthesis is independent of sterol homeostasis regulation.

The effect of low-concentration Pb-acetate (0.1%) on the jejunal ultrastructure has been studied in young male rats. The villi of jejunum of rats exposed to Pb for 30 days had a rough appearance on the surface, which could be associated with a distortion of glycocalyx layer. Areas of extensive degenerative lesions were also observed on the surface of most villi on the 60th day of exposure. All intestinal epithelial cells exhibited various degrees of glycocalyx disturbance, indicating that pronounced toxic effects of Pb were related to modifications of the biochemical properties of the surface coat of the cells.

8.4.10 Genotoxicity and Carcinogenicity

One study has investigated the carcinogenicity of a series of chromate compounds, i.e., Pb-chromate and several Pb-chromate-based compounds. The authors indicated that in this design, Pb-chromate was not carcinogenic, but that 4 of the Pb chromate compounds did induce a very rare tumor in the mice. The remaining five studies focused on Pb-acetate. In most studies, this compound was administered in drinking water at concentrations from 0.5 to 4000 ppm, but one study considered effects from a subcutaneous (SC) injection both in mice and in rats. Consistent with the findings in the 1986 Lead AQCD, Pb not only induced renal tumors, but also induced other tumors (e.g., pituitary, thyroid, testicular), although the possible effect on mammary tumors is difficult to interpret.

Overall, the above studies confirm that Pb is an animal carcinogen and extend our understanding of mechanisms involved to include a role for metallothionein. Specifically, the

recent data show that metallothionein may participate in Pb inclusion bodies and, thus, serves to prevent or reduce Pb-induced tumorigenesis. Much more work is needed to determine the potential exacerbating or ameliorating roles of calcium and selenium and to determine what role Pb-induced immunomodulation may play in the promotion of tumors.

The data currently seem to indicate that Pb can induce anchorage independence in human cells, but its ability to induce neoplastic transformation of human cells is uncertain. Further study of different Pb compounds and the full assessment of their neoplastic potential (i.e., including studies of the ability of treated cells to form tumors in experimental animal models) are needed before definitive conclusions can be drawn.

All together, animal cell culture studies suggest that Pb ions alone cannot transform rodent cells; however, they may be co-carcinogenic or promote the carcinogenicity of other compounds such as chromate.

The proliferative effects of various Pb salts (i.e., Pb-acetate, Pb-chloride, Pb-monoxide, Pb-sulfate), have been evaluated, using liver-derived REL cells. All the Pb compounds tested showed dose- and time-dependent effects on the proliferation of REL cells. Unlike other tumor promoters, Pb compounds did not exhibit effects on cell junctional coupling. Liver hyperplasia induced by Pb-nitrate has been shown to demonstrate sexual dimorphism in all phases of the proliferation as well as in apoptosis.

Investigations of cell cycle-dependent expression of proto-oncogenes in Pb-nitrate (10 μM/100 g body wt)-induced liver cell proliferation showed that peak DNA synthesis occurred at 36 h after a single injection of Pb-nitrate. In addition to DNA synthesis, Pb-induced expression of c-fos, c-myc, and c-Ha-ras oncogenes was also observed in rat liver tissue. Additional studies by the same group reported that Pb-nitrate-induced liver hyperplasia involved an increased expression of c-jun in the absence of c-fos expression.

Differential activation of various PKC isoforms, down regulation of PKC-α, and marked activation of PKC-ϵ in Pb-nitrate-mediated liver hyperplasia suggested the involvement of these PKC enzymes in DNA synthesis and related signal transduction pathways.

The majority of studies on genotoxicity of Pb compounds in animal models focused on mice. Lead was administered by intraperitoneal (IP) or intravenous (IV) injection. Several endpoints were considered, including chromosome aberrations, SCE, micronucleus formation,

and DNA strand breaks. Overall, the results are ambiguous, due in part to study design and the various endpoints considered. The results for SCE are consistently positive.

The potential mutagenicity of Pb compounds in rodent cells was evaluated by using three mutagenesis systems: mutagenesis at the HPRT locus, the gpt locus, and mutations in sodium-potassium ATPase. The results are highly variable and may be specific to the Pb compound considered in each case. In particular, Pb-chromate and Pb-acetate appear to be nonmutagenic. Lead acetate was positive but only at highly cytotoxic concentrations. By contrast, Pb-chloride and Pb-sulfate appeared to be mutagenic at relatively nontoxic concentrations. Insufficient data exist at this point to conclude whether or not Pb is mutagenic in animal cells.

Both Pb-chromate and Pb-nitrate induced DNA-protein crosslinks in cultured mammalian cells. These data suggest that Pb is genotoxic in this manner; however, it is thought that the Pb -chromate-induced DNA-protein crosslinks result from the chromate.

It is plausible that through this mechanism, Pb may act as a co-carcinogen by affecting the metabolism of other chemicals or possibly as a direct carcinogen by enhancing endogenously-induced damage. However, no studies have directly shown that such Pb effects are linked to cancer or alter the potency of another chemical; and, thus, it remains only a plausible hypothesis.

Lead has been classified by IARC as a probable human carcinogen, based mainly on a judgment that there is sufficient animal evidence. This classification is consistent with the National Toxicology Program's Carcinogens Review Committee Report, which recommended that Pb and Pb compounds be considered "reasonably anticipated to be human carcinogens." Ten rat bioassays and one mouse assay have shown statistically significant increases in renal tumors with dietary and subcutaneous exposure to several soluble Pb salts. Animal assays provide reproducible results in several laboratories and in multiple rat strains, with some evidence of multiple tumor sites. Also, short-term studies show that Pb affects gene expression. Similarly, Pb and Pb compounds would likely be classified as likely to be carcinogenic to humans according to the 2005 EPA Guidelines for Carcinogen Risk Assessment.

8.5 KEY LOW-LEVEL LEAD EXPOSURE HEALTH EFFECTS AND IDENTIFICATION OF FACTORS THAT AFFECT SUSCEPTIBILITY TO LEAD TOXICITY

The numerous new studies that have become available since the 1986 Lead AQCD/Addendum and the 1990 Supplement, as discussed above, provide extensive new data on health effects of Pb across a wide exposure range, including information on concentration-response relationships for key Pb-related health effects. Of particular interest for present purposes is the delineation of lowest observed effect levels for those Pb-induced effects that are most clearly associated with blood Pb <10µg/dL in children and/or adults and are, therefore, of greatest public health concern. Tables 8-5 and 8-6 highlight the most important such effects observed in children and adults, respectively, as discussed in the preceding subsections. As evident from those discussions, neurotoxic effects in children and cardiovascular effects in adults are among those best substantiated as occurring at blood-Pb concentrations as low as 5 to 10 µg/dL (or possibly lower); and these categories of effects are currently clearly of greatest public health concern. Other newly demonstrated immune and renal system effects among general population groups are also emerging as low-level Pb-exposure effects of potential public health concern.

The remarkable progress made since the mid-1980s in understanding the effects of Pb on health can be gauged by noting changes that have occurred over time in the questions investigators have addressed. In the 1980s, the question of interest was often: "Does low-level lead exposure affect health?" The questions asked in recent studies have more often focused on details of the associations, including shapes of concentration-response relationships (especially at levels well within the range of general population exposures); biological and socioenvironmental factors that either increase or decrease an individual's risk; prognoses pertinent to Pb-associated effects, efficacy of interventions to reduce adverse effects, and so on. In fact, "low-level," a term long-used to describe exposures that are not sufficiently high to produce clinical signs and symptoms, is increasingly being recognized as a descriptor that has little biological meaning and is interpretable only in a specific historical context. What was considered "low" in the 1980s is an order of magnitude higher than the current mean blood Pb level in the U.S. population, and the current mean remains perhaps as much as two orders of magnitude above preindustrial "natural" background levels in humans. The current CDC screening guideline for children of

Table 8-5. Summary of Lowest Observed Effect Levels for Key Lead-Induced Health Effects in Children

Lowest Observed Effect Blood Lead Level	Neurological Effects	Hematological Effects	Immune Effects
30 µg/dL		Increased urinary δ-aminolevulinic acid	
15 µg/dL	Behavioral disturbances (e.g., inattention, delinquency) Altered electrophysiological responses	Erythrocyte protoporphyrin (EP) elevation	
10 µg/dL	Effects on neuromotor function CNS cognitive effects (e.g., IQ deficits)	Inhibition of δ-aminolevulinic acid dehydratase (ALAD) Pyrimidine-5′-nuclotidase (Py5N) activity inhibition	Effects on humoral (↑ serum IgE) and cell-mediated (↓ T-cell abundance) immunity
5 µg/dL	↓	↓	
	(???)*	(???)*	
0 µg/dL			

*Note: Arrows depict cases where weight of overall evidence strongly substantiates likely occurrence of type of effect in association with blood-Pb concentrations in range of 5-10 µg/dL, or possibly lower, as implied by (???). Although no evident threshold has yet been clearly established for those effects, the existence of such effects at still lower blood-Pb levels cannot be ruled out based on available data.

Source: Adapted/updated from Table 1-17 of U.S. Environmental Protection Agency (1986a).

Table 8-6. Summary of Lowest Observed Effect Levels for Key Lead-Induced Health Effects in Adults

Lowest Observed Effect Blood Lead Level	Neurological Effects	Hematological Effects	Cardiovascular Effects	Renal Effects
30 µg/dL	Peripheral sensory nerve impairment	Erythrocyte protoporphyrin (EP) elevation in males		Impaired Renal Tubular Function
20 µg/dL	Cognitive impairment			
15 µg/dL	Postural sway	Erythrocyte protoporphyrin (EP) elevation in females Increased urinary δ-aminolevulinic acid		
10 µg/dL		Inhibition of δ-aminolevulinic acid dehydratase (ALAD)	Elevated blood pressure ↓	
5 µg/dL			(???)*	Elevated serum creatine (↓ creatine clearance)
0 µg/dL				

*Note: Arrows depict cases where weight of overall evidence strongly substantiates likely occurrence of type of effect in association with blood-Pb concentrations in range of 5-10 µg/dL, or possibly lower, as implied by (???). Although no evident threshold has yet been clearly established for those effects, the existence of such effects at still lower blood-Pb levels cannot be ruled out based on available data.

Source: Adapted/updated from Table 1-16 of U.S. Environmental Protection Agency (1986a).

10 µg/dL is not a "bright line" separating toxicity from safety, but merely a risk management tool. There is no level of Pb exposure that can yet be identified, with confidence, as clearly not being associated with some risk of deleterious health effects. Recent studies of Pb neurotoxicity in infants have observed evidence of effects at population mean blood-Pb levels of only 1 or 2 µg/dL and some cardiovascular, renal, and immune outcomes have been seen at blood-Pb levels below 5 µg/dL. Public health interventions have resulted in declines, over the last 25 years, of more than 90% in the mean blood-Pb level within all age and gender subgroups of the U.S. population, substantially decreasing numbers of individuals at risk for Pb toxicity.

Recent studies have strengthened the consensus that the developing nervous system is the organ system that is probably most sensitive to Pb toxicity in children. Based on new findings, notable neurobehavioral deficits appear to occur at distinctly lower levels of exposure than had been previously documented. The discussion in Section 8.5.1 below focuses on the functional form of these observed relationships and their potential public health implications, starting with blood-Pb/IQ relationships. Probably the most clearly established other Pb effects of concern are cardiovascular effects, with several well-conducted new studies providing strong evidence that elevations in blood Pb levels (even at <10 µg/dL) are significantly associated with increased systolic and diastolic blood pressure in adults (as discussed in Sections 6.5 and 6.10.8.2).

8.5.1 Concentration-Response Relationships for Neurotoxicity Effects

Newly accumulating data validate well the statement made in the 1996 AQCD/Addendum and the 1990 Supplement that adverse effects occur at blood Pb levels of 10 to 15 µg/dL or "possibly lower." In a recent study of 6 to 16 year old children in the NHANES III survey, concentration-related deficits in reading and arithmetic scores were found even when analyses were restricted to children with concurrent blood Pb levels below 5 µg/dL (Lanphear et al., 2000), although these analyses were limited by the fact that direct adjustments could not be made for certain important potential confounding factors (i.e., maternal IQ or caretaking quality in the home) whose inclusion in regression models often notably reduces the size of the Pb coefficient. Canfield et al. (2003a) applied semi-parametric models with penalized splines to their data, essentially allowing the data to reveal the functional form that best described them. These analyses showed that the IQ decline per µg/dL increase in blood Pb was greater below 10 µg/dL than it was above 10 µg/dL. The estimated slope of the IQ decline per µg/dL was

greatest among children for whom the maximum blood Pb level measured over the course of the study never exceeded 10 µg/dL. Also, a similarly steeper slope was seen at lower than at higher blood Pb levels in a re-analysis of the Boston prospective study (Bellinger and Needleman, 2003).

Identifying the functional form that best fits a particular set of data and that presumably represents the best description of the pertinent underlying concentration-response relationship is clearly important. The linear model (Figure 8-6), as the name implies, is linear over the entire range of the exposure data. For certain tests, the assumption is made that the residuals (observed – predicted response) are normally distributed with constant variance, but violations of this assumption in the presence of heteroscedasticity have no real effect on the estimation and minimal effect on the tests of significance. If heteroscedasticity is present but all other conditions are met, the regression model still yields unbiased estimators, but the standard errors can be larger than when remedial efforts such as using weighted regression are employed. The use of regression requires no assumption concerning the distribution of the independent variable (i.e., Pb exposure marker).

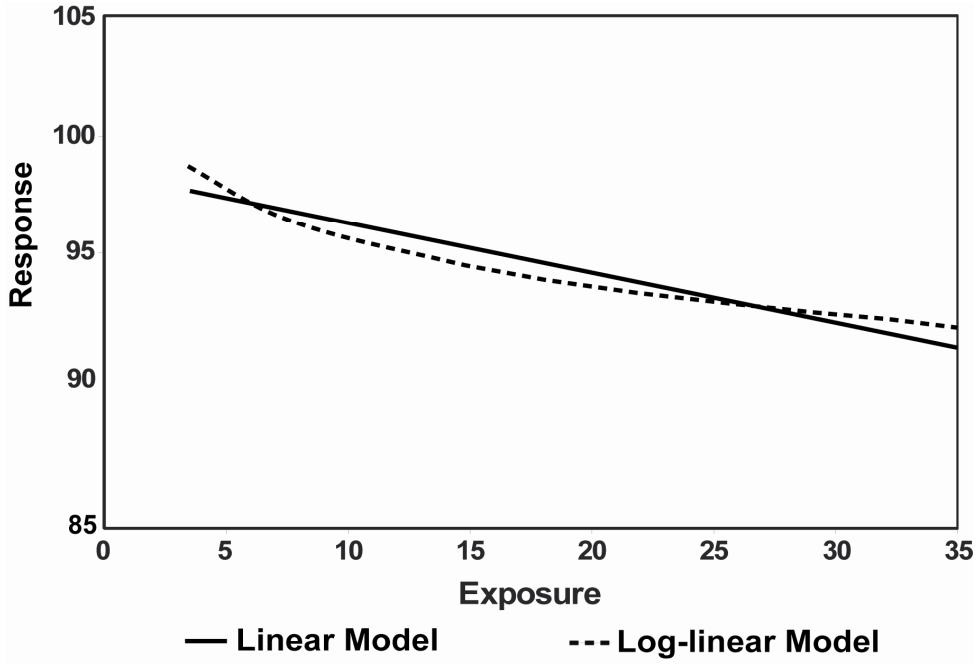


Figure 8-6. Comparison of a linear and log-linear model to describe the relationship between exposure and response.

However, when the form of the heteroscedasticity is an increase in variance with blood Pb level and when the data are lognormally distributed or otherwise skewed, there are possibly a large number of influential data points at high blood Pb where the data are least reliable. In this case, a log transformation of blood-Pb values may result in more precise estimation of the slope parameter. The log-linear model is concave upwards (assuming that the estimated coefficient is negative). It approaches a linear function for very high exposure values, but approaches infinity at very low exposure values. In other words, it implies that the adverse effect of Pb is greater at lower than at higher blood-Pb levels. Blood Pb levels have been shown repeatedly to follow a lognormal distribution (Azar et al., 1975; Billick et al., 1979; Hasselblad and Nelson, 1975; Hasselblad et al., 1980; U.S. Environmental Protection Agency, 1986a; Yankel et al., 1977), but this is not an argument for choosing the log-linear model. The choice of either log-linear or linear may be based on the Akaike's Information Criteria (Akaike, 1973), J-test (Davidson and MacKinnon, 1981), or other statistical tests if the choice is to be based on the best fitting model. Rothenberg and Rothenberg (2005) compared the linear Pb model with the log-linear Pb model for the pooled data from Lanphear et al. (2005) using the J-test. The J-test showed that the log Pb specification was still significant ($p = 0.009$) in a model that also included the linear Pb specification, indicating that the log Pb specification described the data significantly better than did the linear Pb specification. Other models have been used, such as nonparametric models, spline functions, and polynomial models, but the vast majority of the analyses have used either a linear model or a log-linear model.

In a recent publication, Bowers and Beck (2006, p. 520) concluded that "a supralinear slope is a required outcome of correlations between a data distribution where one is lognormally distributed and the other is normally distributed." The authors' analyses were based on three assumptions: that blood lead concentrations are lognormally distributed; that IQ is normally distributed; and that the two have an inverse relationship. However, the authors' conclusions are true only if those assumptions are met. In fact, IQ scores have not been forced into a normal distribution in the epidemiologic analyses. Four of the seven studies included in the pooled analysis by Lanphear et al. (2005) used IQ scores based on the WISC test, and these scores were not normalized. Canfield et al. (2003), in one of the major studies cited by Bowers and Beck (2006), used data from the Stanford-Binet test, but have also stated that the IQ data were not normalized in their analyses(Canfield, pers. comm., September 8, 2006). In addition, the usual

assumption of regression analysis is that the outcome distribution is normal conditional on the predictors, unlike the assumption made by Bowers and Beck (2006) that the outcome is normally distributed. Blood Pb, socioeconomic status and other variable have skewed distributions; when the outcome is linearly related to these predictors, the outcome distribution will be skewed. Therefore, while the conclusions drawn by Bowers and Beck (2006) may be true under certain conditions, their assumptions are not generally the case in the epidemiologic analyses.

In a response to the report by Bowers and Beck (2006), Hornung et al. (2006) provided evidence that the IQ data used in the pooled analysis of seven studies by Lanphear et al. (2005) were not normalized. They state that for the individual studies, a linear relationship between IQ and blood Pb provided an adequate fit over the narrower range of Pb values (<10 µg/dL) associated with each study. In the pooled analysis, the authors tested several different models, and concluded that a loglinear model (a linear relationship between IQ and the log of blood Pb) provided the best fit (Hornung et al., 2006).

The segmented line model consists of joined straight line segments, where the joined points are chosen to best fit the data. The log-linear and the quadratic models have been shown in several cases to better fit the biomarker-response relationship than the linear model. However, these models are not considered practicable for extrapolation outside the range of the biomarker variable. The segmented line model is suggested as a more reasonable model for extrapolation into the low-concentration sparse-data region.

A biological mechanism for a steeper slope at lower than at higher blood-Pb levels has not been identified. It is conceivable that the initial neurodevelopmental lesions at lower Pb levels may be disrupting different biological mechanisms than the more severe effects of high exposures that result in symptomatic poisoning or frank mental retardation (Dietrich et al., 2001). Perhaps the predominant mechanism at very low blood-Pb levels is rapidly saturated, but a different, less rapidly saturated process becomes predominant at blood-Pb levels >10 µg/dL. As Kordas et al. (2006) states, this might help explain why, within the range of exposures not producing overt clinical effects, an increase in blood Pb beyond a certain concentration might cause less additional impairment in children's cognitive functions. However, one must take care not to interpret this as meaning that higher blood-Pb levels do not induce further toxic harm. For example, blood-Pb levels >70-80 µg/dL are still associated with encephalopathy and notable risk for fatal outcome.

The nonlinear concentration-response relationship observed between blood-Pb levels and IQ in recent epidemiologic studies does not preclude the presence of a threshold. Patterson et al. (1991) determined Pb concentrations in the tooth enamel, femur, and rib from buried skeletons of Pre-Columbian Southwest American Indians. They found that the mean natural body burden of adults Homo sapiens uncontaminated by technological Pb was 40 µg Pb/70 kg, which is about one-thousandth of the mean body burden of present day American adults with no occupational exposures and no record of childhood lead poisoning. This suggests that much reduced blood-Pb levels in the 1-10 µg/dL range are still orders of magnitude above pre-industrial natural levels. Thus, a threshold for Pb neurotoxic effects may exist at levels distinctly lower than the lowest exposures examined in these epidemiologic studies.

8.5.2 Persistence/Reversibility of Lead Neurotoxic Effects

Persistence or apparent "irreversibility" of effects can result from two different scenarios: (1) organic damage has occurred without adequate repair or compensatory offsets, or (2) exposure somehow persists. As Pb exposure can also derive from endogenous sources (e.g., bone), a performance deficit that remains detectable after external exposure has ended, rather than indicating irreversibility, could reflect ongoing toxicity due to Pb remaining at the critical target organ or Pb deposited at the organ post-exposure as the result of redistribution of Pb among body pools.

The persistence of effect appears to depend on the duration of exposure as well as other factors that may affect an individual's ability to recover from an insult. The likelihood of reversibility also seems to be related, at least for the adverse effects observed in certain organ systems, to both the age-at-exposure and the age-at-assessment. In occupationally-exposed adults, the central and peripheral nervous system correlates of higher Pb burdens appear to attenuate if exposure is reduced.

Data from the Treatment of Lead Exposed Children (TLC) study, a randomized controlled trial of late outcomes of children treated for Pb poisoning (baseline blood Pb of 20 to 44 µg/dL), support the hypothesis that the deficits associated with exposures of such magnitude are persistent (Dietrich et al., 2004; Rogan et al., 2001). At 36-months post-treatment and at age 7 years, no significant differences in cognition or behavior were noted between the succimer and placebo groups. Current blood Pb levels were significantly associated with cognitive

performance at baseline, 36-months post-treatment, and at 7 years of age, and the regression coefficients were similar in magnitude to those estimated in observational studies (i.e., ~3 point IQ decline per 10 µg/dL increase in blood Pb), providing a linkage between the results of the observational studies and those of this experimental study. However, within-child analyses indicated that changes in developmental test scores over time were not consistently associated with changes over time in blood Pb level.

The prospective studies of childhood Pb exposure, using serial measurements of Pb biomarkers and health outcomes, provide the best opportunities available to assess the natural history of adversities associated with low-level Pb exposures. In some prospective studies, associations observed in infancy between biomarkers of prenatal Pb exposure and slowed neurodevelopment appeared to be attenuated by the time children reached preschool age. It can be difficult to determine, however, whether this reflects actual disappearance of the effect or an increased difficulty in detecting it due to the emergence of associations between Pb biomarkers measured postnatally and neurodevelopment. It is notable, however, that in some prospective studies of children, associations between biomarkers of prenatal Pb exposure and various outcomes in middle adolescence have been reported, suggesting that the persistence of the associations might be endpoint-specific. For example, among children in Kosovo, Yugoslavia, IQ scores at the age of 8 years were inversely associated with a composite index of prenatal Pb exposure (average of mothers' blood Pb levels at midpregnancy and at delivery) (Wasserman et al., 2000b). This association was independent of changes in postnatal blood Pb levels. Or, among 15 to 17 year old inner-city children in Cincinnati, OH, maternal blood-Pb levels (ranging from 1 to ~30 µg/dL) in the first trimester were inversely related to attention and vasoconstriction (Ris et al., 2004) and positively related to the frequency of self-reported delinquent behaviors (Dietrich et al., 2001).

In most prospective cohort studies, the potential for true longitudinal analysis of the data has not been fully exploited, with the data evaluated in what is effectively a series of cross-sectional analyses. Nevertheless, the results of the prospective studies are consistent in showing that higher postnatal Pb biomarkers are associated with neurocognitive deficits that persist, in some studies, into early adulthood when the concurrent Pb exposures are generally much lower. Ongoing external exposure does not appear to be necessary to maintain the deficits, although, as noted previously, it is not possible to exclude entirely a role for ongoing endogenous exposures

of the target organs resulting from the redistribution, over time, of Pb stores among different compartments. These data are consistent with those from experimental nonhuman primate studies, in which the temporal characteristics of exposure are manipulated as opposed to merely observed, as in the human studies.

One study examined the persistence of lead-related cognitive impairment using an intervention that resulted in a marked reduction in external Pb exposure (Soong et al., 1999). The cognitive abilities of exposed children (n = 32, median blood-Pb level of 15.1 µg/dL [range 7.7-31.7]) from a kindergarten located near a Pb-recycling plant in Taiwan were compared to a referent group of children (n = 35, median blood-Pb of 8.4 µg/dL [range 4.8-12.8]) from another kindergarten 5 km away from the plant. Both groups of children were comparable with respect to age, sex, birth order, sibling number, and parental education level. The exposed children were found to have significantly lower IQ levels compared to the referent children, with a median score of 94.5 points (range 60-121) compared to 101 points (range 76-129). The next year, the school located near the Pb-recycling plant was moved an additional 2 km away. A follow-up study was conducted with 28 in each group 2½ years later. The median blood-Pb levels of the previously exposed and referent children decreased to 8.5 µg/dL (range 5.0-15.0, average decline of 6.9 µg/dL) and 7.0 µg/dL (range 4.0-11.0, average decline of 1.7 µg/dL), respectively. The average IQ scores in the previously exposed children increased by 11.7 points (SD 13.2), with a median value of 107 points (range 75-135). This value was not significantly different from the median score of the referent children, 109.5 points (range 79-132). These results indicate that IQ impairment resulting from blood-Pb elevations for a period of 1 to 3 years in 3 to 5 year old children was at least partially reversible when external Pb exposure was reduced.

Only limited data are available on factors that influence the likelihood that an association observed between an early Pb biomarker and later outcome will persist among children. In one study, the association between prenatal exposure and cognitive development in infancy and the preschool period appeared to attenuate among children living in more privileged circumstances or in whom postnatal Pb exposures were lower (Bellinger et al., 1988, 1990). These findings are consistent with those from cross-sectional epidemiologic studies showing that the effects of a given level of Pb exposure are more severe among disadvantaged children (Lansdown et al., 1986; Winneke and Kraemer, 1984) and from experimental animal studies showing that being

raised in an enriched environment can reduce the apparent detrimental impact of Pb exposure on learning (Guilarte et al., 2003; Schneider et al., 2001).

8.5.3 Factors Affecting Susceptibility to Lead Toxicity

Although increased Pb exposure has been linked to adverse health effects in many different organ systems, scatterplots reveal tremendous variability of observed points about the best fit lines representing the concentration-response relationships. In other words, individuals for whom the Pb biomarker measured has the same value can have markedly different values on the health indicator measured. Even for neurobehavioral deficits in children, the correlation between biomarker level and test score rarely exceeds 0.2, indicating that the explained variance in the test score generally does not exceed 5%. A major challenge is therefore to decompose this variability, to distinguish components of it that reflect error from components that reflect biological processes that determine an individual's response to Pb.

Deviation of the observed points from the fitted point can have many sources. Exposure misclassification is one source. The Pb biomarker measured might not adequately capture the Pb dose delivered to the target organ that, at the time, is most appropriate biologically. In general, the error would be expected to be non-differential, i.e., it would not introduce a systematic bias in the estimation of the concentration-response relationship. On average, such misclassification would be expected to result both in an attenuation of the slope of the concentration-response relationship and an increase in the scatter of the observations. As focus shifts to the risks associated with lower and lower levels of Pb exposure, the importance of errors introduced by poor dosimetry will assume greater importance insofar as the effects at such levels will presumably be more subtle and increasingly difficult to detect amid the noise contributed by exposure misclassification. Outcome misclassification is another source of error that is likely to contribute to apparent interindividual variability in response. This results if the indicator of the critical health effect that is measured is fallible, i.e., an imperfect measure of the target function. Such misclassification would generally be expected to be non-differential, introducing random noise rather than a systematic bias.

Another likely source of scatter in observed points is true interindividual variability in response to a given Pb dose. That is, the magnitude of individual response to Pb might depend on other characteristics of that individual. Three major categories of such effect modifying

factors that might influence susceptibility to Pb toxicity are genetic polymorphisms, nutritional status, and social environmental factors. Adequate data are not available to provide a quantitative estimate of the amount of interindividual variability in susceptibility to Pb.

Influence of Genetic Polymorphisms on Risk

Genetic polymorphisms that are presumed to influence Pb toxicokinetics and/or toxicodynamics have been identified, mostly in studies of adults who were occupationally exposed to Pb. The magnitude of Pb-associated renal dysfunction appears to vary, in complex ways, with the delta-aminolevulinic acid dehydratase (ALAD) polymorphism (Chia et al., 2005, 2006). Lead workers with the ATP1A2(3′) polymorphism appear to be at increased risk of Pb-associated effects on blood pressure (Glenn et al., 2001). The slope of the association between floor dust-Pb and blood-Pb is steeper among children with the less common variant of the vitamin D receptor (Fok 1 or B) than among children with the wild-type allele (Haynes et al., 2003). In adults, these same alleles are associated with higher blood-Pb levels and increased blood pressure (Schwartz et al., 2000a; Lee et al., 2001). Greater Pb-associated reductions in renal function have been observed in adults with a variant allele of nitric acid synthetase, although cardiovascular outcomes, such as blood pressure and hypertension do not appear to depend on the eNOS (endogenous nitric oxide synthase) allele (Weaver et al., 2003b). Adults with variants of the hemochromatosis gene (C282Y and/or H63D) have higher patella Pb levels (Wright et al., 2004). With regard to polymorphisms that modify Pb neurotoxicity, workers with the apolipoprotein E4 allele showed greater Pb-associated decreases in neurobehavioral function than did workers with the E1, E2, or E3 alleles (Stewart et al., 2002). Chia et al. (2004) speculated that the ALAD2 confers protection against Pb neurotoxicity, although Kamel et al. (2003) reported that this variant allele is associated with an increased risk of amyotrophic lateral sclerosis. This work is in its early stages and, while it promises to shed light on bases of susceptibility to Pb toxicity, firm conclusions cannot yet be drawn.

Influence of Nutritional Status on Risk

Only limited epidemiologic data are available on the role of nutritional status in modifying an individual's risk of Pb toxicity. Adjusting for severity of environmental Pb contamination, iron-deficient children appear to have higher blood-Pb levels than iron-replete

children (Bradman et al., 2001). One interpretation of these data is that children experiencing the same external Pb dose can experience different internal doses. In another study of iron status, a decline in blood-Pb level was associated with improved cognitive performance in iron-sufficient but not in iron-deficient children (Ruff et al., 1996). Among the possible explanations for this finding is that iron deficiency contributes to pharmacodynamic variability, increasing the toxicity of a given Pb dose. Some evidence suggests that the intellectual deficit associated with an elevated blood-Pb level is greater among undernourished children than well-nourished children (Gardner et al., 1998).

Several studies have suggested that dietary calcium may have a protective role by decreasing absorption of Pb in the gastrointestinal tract and decreasing the mobilization of Pb from bone stores to blood, especially during periods of high metabolic activity of the bone such as pregnancy and lactation. Lower calcium intake during pregnancy, especially the second half, appears to increase the mobilization of Pb from bone compartments (Hernandez-Avila et al., 1996). However, in other studies, calcium supplementation had no effect on bone-Pb levels in pregnant and lactating women (Rothenberg et al., 2000; Téllez-Rojo et al., 2002).

Influence of Health Status on Risk

The influence of an individual's health status on susceptibility to Pb toxicity has been demonstrated most clearly for renal outcomes. Individuals with diabetes, hypertension, and chronic renal insufficiency are at increased risk of Pb-associated declines in renal function, and indications of altered kidney function have been reported at blood Pb levels ranging somewhat below 5 µg/dL (Lin et al., 2001, 2003; Muntner et al., 2003; Tsaih et al., 2004). As noted in the previous section, children with nutritional deficiencies also appear to be more vulnerable to Pb-associated neurobehavioral deficits.

Influence of Coexposures on Risk

Epidemiologic studies do not provide an adequate basis for determining whether cigarette smoking and/or alcohol affect the nature or severity of Pb health effects. Both factors have often been included in models of both child and adult health outcomes to adjust for potential confounding. Both have also been evaluated as pertinent pathways of adult exposure. However, their possible roles as effect modifiers have not been well studied.

Although most individuals are not exposed to Pb in isolation but rather to Pb in combination with other toxicants (e.g., cadmium, arsenic, mercury, and polychlorinated biphenyls), epidemiologic studies have generally focused solely on Pb. Other toxicant exposures have sometimes been measured but are usually treated as potential confounders in the statistical analyses, with their potential as possible modifiers of Pb toxicity left unexplored (Bellinger, 2000). Thus, available epidemiologic studies do not provide an adequate basis for determining the extent to which co-exposure to other toxicants may affect the nature or severity of Pb-related health effects.

Influence of Timing of Exposure on Risk

Children

Available studies do not provide a definitive answer to the question of whether Pb-associated neurodevelopmental deficits are the result of exposure during a circumscribed critical period or of cumulative exposure. Although support can be cited for the conclusion that it is exposure within the first few postnatal years that is most important in determining long-term outcomes (Bellinger et al., 1992), other studies suggest that concurrent blood-Pb level is as predictive, or perhaps more predictive, of long-term outcomes than are early blood-Pb levels (Canfield et al., 2003a; Dietrich et al., 1993a,b; Tong et al., 1996; Wasserman et al., 2000b). Because of the complex kinetics of Pb, an accumulative toxicant, it is extremely difficult to draw strong conclusions from these observational studies about windows of heightened vulnerability in children. The high degree of intra-individual "tracking" of blood Pb levels over time, especially among children in environments providing substantial, chronic exposure opportunities (e.g., residence near a smelter or in older urban dwellings in poor repair), poses formidable obstacles to identifying the time interval during which exposure to Pb caused the health effects measured in a study. It could be that damage occurred during a circumscribed period when the critical substrate was undergoing rapid development, but that the high correlation between serial blood Pb levels impeded identification of the special significance of exposure at that time.

Under such circumstances, an index of cumulative blood Pb level or concurrent blood Pb level, which might be a good marker of overall body burden under conditions of relatively steady-state exposure, might bear the strongest association with the effect. Under these circumstances, however, it might be incorrect to conclude that it was the later exposures,

incurred around the time that the effect was detected, that was responsible for producing it. While some observations in children as old as adolescence indicate that exposure biomarkers measured concurrently are the strongest predictors of late outcomes, the interpretation of these observations with regard to critical windows of vulnerability remains uncertain. Additional research will be needed to distinguish effects that reflect the influence of later Pb exposures from effects that reflect the persistent of effects resulting from exposure during some prior critical window. Resolving this issue solely on the basis of data from observational studies will be difficult due to the high intercorrelation among blood Pb measures taken at different ages.

Increasing attention is being devoted to determining the extent to which early childhood Pb exposures increases the risk of adverse effects that are only apparent at older ages (i.e., delayed or latent effects). Among young adults who lived as children in an area heavily polluted by a smelter and whose current Pb exposure was low, higher bone Pb levels were associated with higher systolic and diastolic blood pressure (Gerr et al., 2002). In adult rats, greater early exposures to Pb are associated with increased levels of amyloid protein precursor, a marker of risk for neurodegenerative disease (Basha et al., 2005).

Aging Population

Increases in blood Pb for postmenopausal women have been attributed to release of Pb from the skeleton associated with increased bone remodeling during menopause in both occupationally- and environmentally-exposed women (Garrido-Latorre et al., 2003; Popovic et al., 2005). Also, in middle-aged to elderly males from the Normative Aging Study, patella Pb accounted for the dominant portion of variance in blood Pb (Hu et al., 1996). These findings suggest that the skeleton serves as an endogenous source of Pb in the aging population.

Considerable evidence also suggests that indicators of cumulative or long-term Pb exposure are associated with adverse effects in several organ systems, including the central nervous, renal, and cardiovascular systems. Among occupationally-exposed men, higher tibia Pb levels have been associated with increased cognitive decline over repeated assessments (Schwartz et al., 2005). With regard to the renal system, increased Pb exposure may accelerate the effects of normal aging, producing a steeper age-related decline in function. Weaver et al. (2003a) observed that higher Pb exposure and dose were associated with worse renal function in older workers, but with lower blood urea nitrogen and serum creatinine in young workers.

Pregnancy

Mobilization of Pb from the skeleton also occurs during pregnancy and lactation due to increased bone remodeling to meet the calcium requirements of the developing fetus (Hertz-Picciotto et al., 2000; Manton, 1985; Silbergeld, 1991). In women who have been exposed to Pb in childhood and have accumulated large stores in their bones, there may be significant mobilization of Pb from bone to blood during late pregnancy and lactation. Lead isotope studies on immigrant women to Australia reported increases of 20% to 99% during pregnancy (Gulson et al., 1997, 1998). Skeletal Pb contribution to blood Pb was significantly greater during the postpregnancy period than during the second and third trimesters. The highest probability of Pb toxicity for the mothers will be in postpartum while they are lactating; the infants will be particularly vulnerable during the prenatal period, especially in the last weeks of pregnancy (Manton et al., 2003). Calcium supplementation appears to provide a modest reduction in blood-Pb levels in pregnant or lactating women (Gulson et al., 2004; Hernandez-Avila et al., 2003).

A variety of adverse reproductive outcomes have been associated with higher paternal or maternal Pb exposures, including reduced fertility, spontaneous abortion, gestational hypertension, congenital malformations, fetal growth deficits, and neurobehavioral deficits in offspring. The levels of exposure at which different adverse outcomes occur vary. Increased risks of spontaneous abortion, neurobehavioral deficits in offspring and, in some studies, gestational hypertension, have been reported at pregnancy blood Pb levels below 10 µg/dL (Bellinger, 2005).

8.6 POTENTIAL PUBLIC HEALTH IMPLICATIONS OF LOW-LEVEL LEAD EXPOSURE

8.6.1 Introduction

In studies of Pb toxicity, health endpoints have more often been continuously-distributed indices such as blood pressure or IQ. A view that the endpoints should be diagnoses rather than measured values on the underlying indices is that a change in the value of a health index that does not exceed the criterion value defining the diagnosis is therefore without consequence for an individual's health. The World Health Organization (WHO) definition of "health,"

is: "Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (World Health Organization, 1948). By this definition, even decrements in health status that are not severe enough to result in the assignment of a diagnosis might be undesirable if they reflect a decrement in an individual's well-being but are not severe enough to meet diagnostic criteria. Deficits in health indices or well-being may not be observable except in aggregate, at the population level. The American Thoracic Society discusses similar concepts of shift in population distribution and health effects (American Thoracic Society, 2000).

Sometimes, the importance of a Pb-associated change on a health index is evaluated by comparing it to the standard error of measurement of the index, i.e., the statistic that defines the range within which an individual's "true" value on the index is likely to lie. For instance the standard error of measurement for full scale IQ is 3 to 4 points, leading some to conclude that the estimated IQ decrement of 3 points per 10 μg/dL increase in blood Pb level is "in the noise" of measurement and, therefore, meaningless. A similar claim has been made with regard to the magnitude of the association between Pb and blood pressure. The error in this argument is that the estimated decrement of 3 IQ points per 10 μg/dL applies to grouped, not individual, data. For measurement error to provide an explanation for the observation of an association that is approximately the size of the standard error of measurement, it would be necessary to postulate that the true association is null, but that, by chance or because of some bias, the measured IQ scores of the individuals with higher Pb exposures were systematically underestimated (i.e., their true IQ scores lie in the upper tails of the 95% CI for the children's observed scores) and that the measured IQ scores of the individuals with lower exposures were systematically overestimated (i.e., their true IQ scores lie in the lower tails of the 95% CI). Thus, this argument requires an assumption that the direction of measurement error is highly correlated with exposure status. The fundamental flaw is using a statistic that pertains to individual-level data to draw inferences about group-level data.

Nosology (the classification and naming of diseases) is dynamic as knowledge accrues. The total serum cholesterol level that is considered indicative of hyperlipidemia has dropped steadily over the past 40 years. Second, even within the range of health index values that are sub-diagnostic, variations on the index are significantly associated with health outcomes. For instance, even among children with birth weights greater than the cut-off used to define

"low birth weight," birth weight is significantly associated with IQ at age 7 years (Matte et al., 2001). Third, exposure-related changes on a health index can be markers or indicators of other changes that are likely to have occurred whose significance is more certain. For instance, slower completion of a commonly-used neuropsychological test, the Grooved Pegboard, is associated with poorer handwriting, and reduced ability to copy a drawing is associated with a greater risk of a need for remedial school services (Bellinger, 2004).

The critical distinction between population and individual risk, an issue pertinent to many questions in chronic disease epidemiology, has often been blurred in discussions of the public health implications of Pb-associated decrements in health. In regard to neurodevelopment, although a two- or three-point decline in IQ might not be consequential for an individual, it is important to recognize that this figure represents the central tendency of the distribution of declines among individuals. Thus, some individuals might manifest declines that are much greater in magnitude, while others manifest no decline at all, reflecting interindividual differences in vulnerability. Moreover, the import of a decline for an individual's well-being is likely to vary depending on the portion of the IQ distribution. For an individual functioning in the low range due to the influence of developmental risk factors other than Pb, a Pb-associated decline of several points might be sufficient to drop that individual into the range associated with increase risk of educational, vocational, and social failure.

The point estimate indicating a modest mean change on a health index at the individual level can have substantial implications at the population level. For example, although an increase of a few mmHg in blood pressure might not be of concern for an individual's well-being, the same increase in the population mean might be associated with substantial increases in the percentages of individuals with values that are sufficiently extreme that they exceed the criteria used to diagnose hypertension (Rose and Day, 1990). In other words, the mean value conveys substantial information about the percentage of individuals with clinically relevant, extreme values of the indicator. Moreover, interventions that shift the population mean, in a beneficial direction, by an amount that is without clinical consequence for an individual have been shown to produce substantial decreases in the percentage of individuals with values that are clinically significant (Bellinger, 2004). The following subsections discuss quantitatively Pb-related effects of a population level change in IQ and blood pressure.

8.6.2 Potential Implications of Lead Effects on Intelligence

The outcome most often examined to investigate the neurotoxic effects of Pb is IQ. Although the definition of "intelligence" is quite abstract, IQ remains a useful outcome measure as it is correlated with important measures of life success, such as academic achievement, earnings, and social status (Bellinger, 2003; Weiss, 2000). Several studies reported quantitative relationships between measures of IQ and current blood Pb levels for children aged 2 to 11 years old. The estimated relationships as reported by the authors are summarized in Table 8-7, organized by the type of model used in the analysis. The pooled analysis by Lanphear et al. (2005) included the studies of Baghurst et al. (1992), Bellinger et al. (1992), Canfield et al. (2003a), Dietrich et al. (1993a), Ernhart et al. (1989) and Wasserman et al. (1997). That pooled analysis also included the Mexico City study of Schnaas et al. (2000). The results from Schnaas et al. (2000) are not included in Table 8-7, because the authors did not provide regression coefficients in their paper, thus the concentration-response relationship was not estimable. The study by Earnhart et al. (1989) also is not included, as slopes cannot be estimated from the covariate variances presented for the adjusted models.

The curves over a range of blood Pb levels from the 10th percentile to the 90th percentile are shown in Figure 8-7. The curves are restricted to that range because log-linear curves become very steep at the lower end of the blood Pb levels, and this may be an artifact of the model chosen. The percentiles are estimated using various methods and are only approximate values. Studies which estimated a linear relationship are shown as reported, and similarly for the log-linear relationships. Note that these are not forest plots of slopes or hazard ratios—they are the actual estimated relationships.

Several conclusions can be drawn from these graphs. First, note that the overall IQ levels are quite different. This results from different populations and from different applications of the IQ tests. Second, all studies showed a decreasing IQ score as the blood-Pb level increased. It is the slope of the studies that is relevant, not the actual IQ scores. Third, for studies with lower blood-Pb levels, the slopes appear to be steeper. This is the reason that many authors choose to use the log-linear model. However, for those studies where the blood-Pb levels were generally high, the log-linear and linear models are almost identical. Thus, it is not surprising that some authors chose a linear model instead of a log-linear model. The curves in Figure 8-7 do not show evidence of a no-effect threshold because the slopes increase as the blood-Pb levels become

Table 8-7. Summary of Studies with Quantitative Relationships for IQ and Blood Lead

Reference	Study Location	n	Age of Children	Model Used	Slope and 95% CI (IQ points/µg/dL) – Blood Lead 10th to 90th Percentile	Slope and 95% CI (IQ points/µg/dL) – Blood Lead 10th Percentile to 10 µg/dL
Bellinger et al. (1992; reanalyzed Bellinger and Needleman, 2003)[a]	Boston, Massachusetts	148	10 years	Linear	−0.6 (−1.0, −0.2)	−1.6 (−2.9, −0.2)
Canfield et al. (2003a)	Rochester, New York	154	5 years	Linear	−0.6 (−1.0, −0.2)	−1.8 (−3.0, −0.6)
Dietrich et al. (1993a)	Cincinnati, Ohio	148	6 years 6 months	Linear	−0.3 (−0.6, −0.1)	NA
Kordas et al. (2006)	Torreón, Mexico	589	6-8 years	Linear	−0.2 (−0.4, 0.1)	−0.4 (−1.2, 0.4)
Lanphear et al. (2005)[b]	International Pooled Analysis	1,333	4 years 10 months to 10 years	Linear	NA	−0.8 (−1.7, 0.1)
Téllez-Rojo et al. (2006)	Mexico City, Mexico	294	24 months	Linear	NA	−1.0 (−1.8, −0.3)
Wasserman et al. (1997)[a]	Kosovo, Yugoslavia	258	7 years	Linear	−0.2 (−0.3, −0.2)	NA
Al-Saleh et al. (2001)[c]	Riyadh, Saudi Arabia	533	6-12 years	Log-linear	−0.7 (−1.2, −0.1)	−0.8 (−1.4, −0.2)
Baghurst et al. (1992)[a]	Port Pirie, South Australia	494	7 years	Log-linear	−0.2 (−0.4, 0.1)	NA
Lanphear et al. (2005)[b]	International Pooled Analysis	1,333	4 years 10 months to 10 years	Log-linear	−0.2 (−0.3, −0.2)	−0.4 (−0.6, −0.3)
Schnaas et al. (2006)	Mexico City, Mexico	150	6-10 years	Log-linear	−0.4 (−0.6, −0.1)	NA
Téllez-Rojo et al. (2006)	Mexico City, Mexico	294	24 months	Log-linear	NA	−0.9 (−1.4, −0.5)

[a] Slope estimates for the relationship between IQ and concurrent blood lead levels are presented, except for Bellinger et al. (1992; reanalyzed Bellinger and Needleman, 2003), which used 24-month blood lead levels; Baghurst et al. (1992), which used lifetime average blood lead levels; and Wasserman et al. (1997), which used lifetime cumulative blood lead levels.

[b] The pooled analysis by Lanphear et al. (2005) included data from seven individual studies, including Baghurst et al. (1992), Bellinger et al. (1992), Canfield et al. (2003a), Dietrich et al. (1993a), and Wasserman et al. (1997).

[c] In Al-Saleh et al. (2001), 69% (n = 368) of the children had blood lead levels <10 µg/dL. The estimated slope for blood lead levels <10 µg/dL is based on the model for the entire sample population.

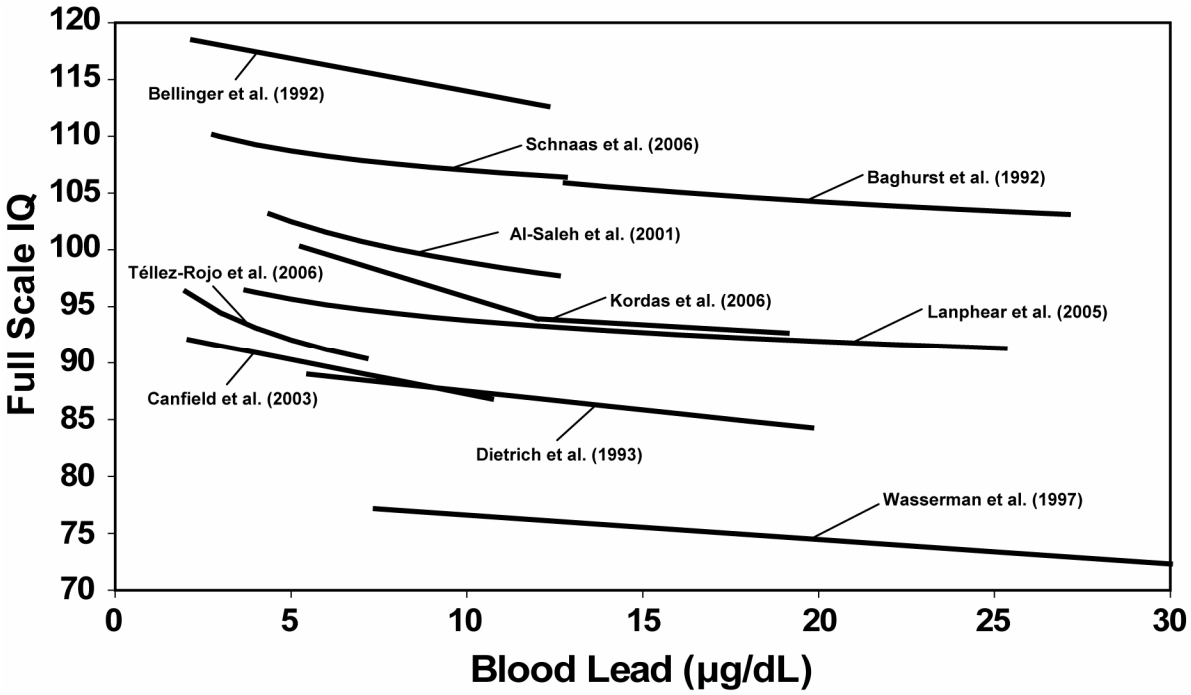


Figure 8-7. Concentration-response relationships of IQ to blood lead for the individual studies and the pooled analysis by Lanphear et al. (2005).

smaller. The observed mean adjusted IQ levels (for blood Pb <5, 5-10, 10-15, 15-20, and >20 µg/dL) reported by Lanphear et al. (2005) also show no evidence of a threshold, as seen in Figure 8-8.

Weiss (1990) predicted, on purely statistical grounds, that a downward shift of five points in mean IQ, if the amount of dispersion in the distribution remained the same, should be accompanied by a doubling of the numbers of individuals with scores two or more standard deviations below the mean and a reduction by half of the number of individuals with scores two or more standard deviations above the mean. With respect to Pb, the general accuracy of this prediction has been empirically demonstrated in two different datasets by Needleman et al. (1982) and Bellinger (2004). An illustrative example is provided below, and it shows further evidence of the change in percentages of individuals with IQ <80 or <70 points and >120 or >130 points after restricting the analysis to those with blood-Pb levels <10 µg/dL.

The slope of −0.9 points/µg/dL was used in these calculations. This slope is the median value from the estimated slopes for blood-Pb levels <10 µg/dL presented in Table 8-7. A nonexposed population was assumed to have a standard mean IQ of 100 and standard

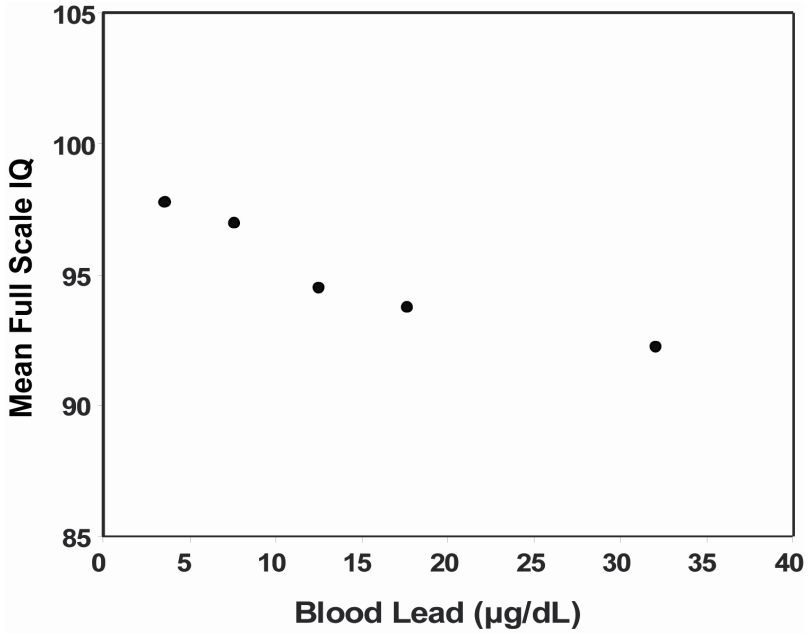


Figure 8-8. Mean blood lead levels adjusted for HOME score, maternal education, maternal IQ, and birth weight from the pooled analysis of seven studies by Lanphear et al. (2005). Mean adjusted IQ levels at blood lead levels of <5, 5 to 10, 10 to 15, 15 to 20, and >20 µg/dL are shown.

deviation of 15 at a blood-Pb exposure of 0 µg/dL. The fraction of the population that would have an IQ <80 or <70 as a function of blood-Pb level was then calculated. The results are shown in Figure 8-9A. The fraction of the population with an IQ level less than 80 more than doubles from 9% with no Pb exposure to 23% with a blood-Pb level of 10 µg/dL. The fraction with an IQ level below 70, a level often requiring community support to live (World Health Organization, 1992) increases from a little over 2% with no Pb exposure to about 8% with a blood-Pb level of 10 µg/dL.

The Pb-related decrements in IQ are manifested fairly uniformly across the range of IQ scores (Needleman et al., 1982). Thus, a shift in the mean value of a health indicator has substantial importance for both extremes of the distribution. In the case of Pb, a downward shift in the mean IQ value is not associated only with a substantial increase in the percentage of individuals achieving very low scores, but also with substantial decreases in percentages achieving very high scores. Based on the study by Bellinger et al. (1987) examining intelligence test scores of Pb-exposed children, Weiss (1988) discussed the shift of the population

distribution of IQ from a mean of 100 and a standard deviation of 15 to a mean of 95, a 5% reduction. When the mean IQ level is 100, 2.3% of the individuals in a given population would score above 130. However, with the population distribution shift and the resulting mean decline in IQ, only 0.99% of the individuals would score above 130. Weiss states that the implication of such a loss transcends the current circumscribed definitions of risk. Similar results were observed using the slope of −0.9 points/ µg/dL to examine the effects on the percentage of individuals with an IQ >120 or >130 points at blood Pb levels <10 µg/dL (Figure 8-9B). The fraction of individuals with an IQ >120 decreased from about 9% with no Pb exposure to less than 3% at a blood Pb level of 10 µg/dL. The fraction of individuals with an IQ >130 points decreased from 2.25% to 0.5% with a blood Pb level change from 0 to 10 µg/dL.

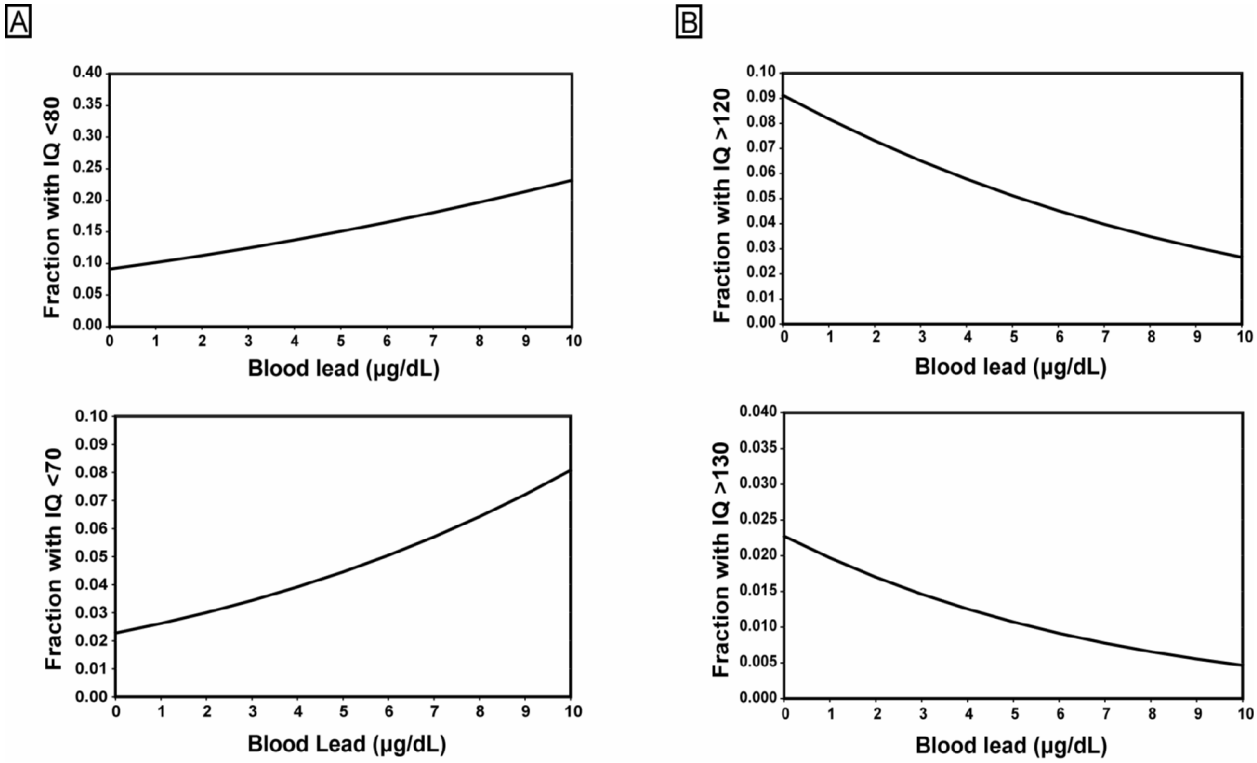


Figure 8-9. Effect of blood lead on fraction of population with IQ levels <80 or <70 points (A) and IQ levels >120 or >130 points (B).

8.6.3 Potential Implications of Cardiovascular Effects of Lead

In human epidemiology studies investigating the cardiovascular effects of Pb, blood pressure has been examined most frequently, as discussed in Section 6.5.2 of Chapter 6. Results from the Framingham Heart Study show that higher levels of blood pressure, even within the nonhypertensive range, impose increased rates of cardiovascular disease (Kannel, 2000a,b). A continuous graded increase in cardiovascular risk is observed as blood pressure increases, with no evidence of a threshold value. Most events arise not in the most severe cases, but mainly in those with high normal blood pressure (i.e., mild hypertension). This view is further supported by the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (Chobanian et al., 2003). Kannel (2000b) states that reducing even moderate elevation in blood pressure is likely to be beneficial.

Kannel (2000a) emphasized that systolic blood pressure exerts a strong influence on more serious cardiovascular events, as it is the prime causal function of hypertension and its adverse cardiovascular sequelae. Cardiovascular events include coronary disease, stroke, peripheral artery disease, and cardiac failure. Risk ratios are larger for cardiac failure and stroke, but coronary disease (i.e., myocardial infarction, angina pectoris, sudden death) is the most common and most lethal sequela of hypertension (Kannel, 1996). Kannel (2000a) notes that the Framingham Heart Study has recognized that elevated blood pressure tends to occur alongside other major risk factors of cardiovascular disease such as glucose intolerance, dyslipidemia, abdominal obesity, and left ventricular hypertrophy, among others. If a cluster of multiple risk factors is present, the hazard is formidable for coronary disease and stroke.

No single critical level for blood pressure is evident. The risk appears to be simply proportional from the lowest to the highest level recorded. In the Multiple Risk Factor Intervention Trial (MRFIT), Neaton et al. (1995) confirmed a continuing and graded influence of systolic blood pressure on cardiovascular disease mortality extending down into the range of <140 mm Hg. The Prospective Studies Collaboration (2002) meta-analysis of 61 prospective studies relates blood pressure to vascular mortality without indication of a threshold down to 115/75 mm Hg. The absence of a demonstrable safe or critical level of blood pressure suggests using the range of blood pressure rather than discrete categories such as hypertension.

Many studies have provided evidence for a relationship between blood Pb and systolic blood pressure. In particular, the meta-analysis of Nawrot et al. (2002) indicated that a doubling

of the blood Pb (e.g., from 5 to 10 μg/dL) corresponded to a 1 mm Hg increase in systolic blood pressure. As noted earlier, although this magnitude of increase in systolic blood pressure is not particularly meaningful clinically for any given individual, a population shift of 1 mm Hg is important.

The Framingham Heart Study results (Kannel, 2000a) were used to estimate a typical population distribution of systolic blood pressure values (Figure 8-10). The distribution of systolic blood pressure values was approximated well by a lognormal distribution for both women and men ($p \geq 0.4$). The relationship between systolic blood pressure and the risk of cardiovascular events was also given by Kannel (2000a), as shown in Figure 8-11. To estimate population risk, it was assumed that the effect of blood Pb on blood pressure was to shift the entire distribution by the amount given by Nawrot et al. (2002). For each shift in the distribution, the entire distribution was integrated out over the risk given in Figure 8-11.

The result estimated was the expected number of cardiovascular events per 1,000 person years, and this was plotted for blood-Pb levels ranging from 5 to 15 μg/dL for both women and men. The results are shown in Figure 8-12. Although the effects are modest, they translate into a large number of events for a moderate population size. For example, a decrease in blood Pb from 10 to 5 μg/dL results in an annual decrease of 27 events per 100,000 women and 39 events per 100,000 men.

In order to relate the effects of blood Pb levels to air Pb concentrations, an estimate of the relationship of air Pb to blood Pb in adults is necessary. One such estimate, as an example, can be derived from the Azar et al. (1975) study, which used personal monitors to estimate air Pb exposure in 149 adults (as discussed in Chapter 11 of the 1986 Lead AQCD). In that study, the estimated slope at an air Pb concentration of 1.0 $\mu g/m^3$ was a 2.57 μg/dL increase in blood Pb per 1 $\mu g/m^3$ increase in air Pb. Based on this slope estimate, a 0.25 $\mu g/m^3$ decrease in air Pb would lead to a 0.64 μg/dL decrease in blood Pb levels. Using both the relationship between blood-Pb levels and blood pressure (i.e., a doubling of the blood-Pb corresponds to a 1 mm Hg increase in systolic blood pressure) and the relationship between blood pressure and cardiovascular events, a decrease of 0.64 μg/dL in blood Pb from 5 μg/dL to 4.36 μg/dL would be projected to lead to an annual decrease of 5 cardiovascular events per 100,000 for women and 8 events per 100,000 for men. For a city of 3 million people (about the size of Chicago) this

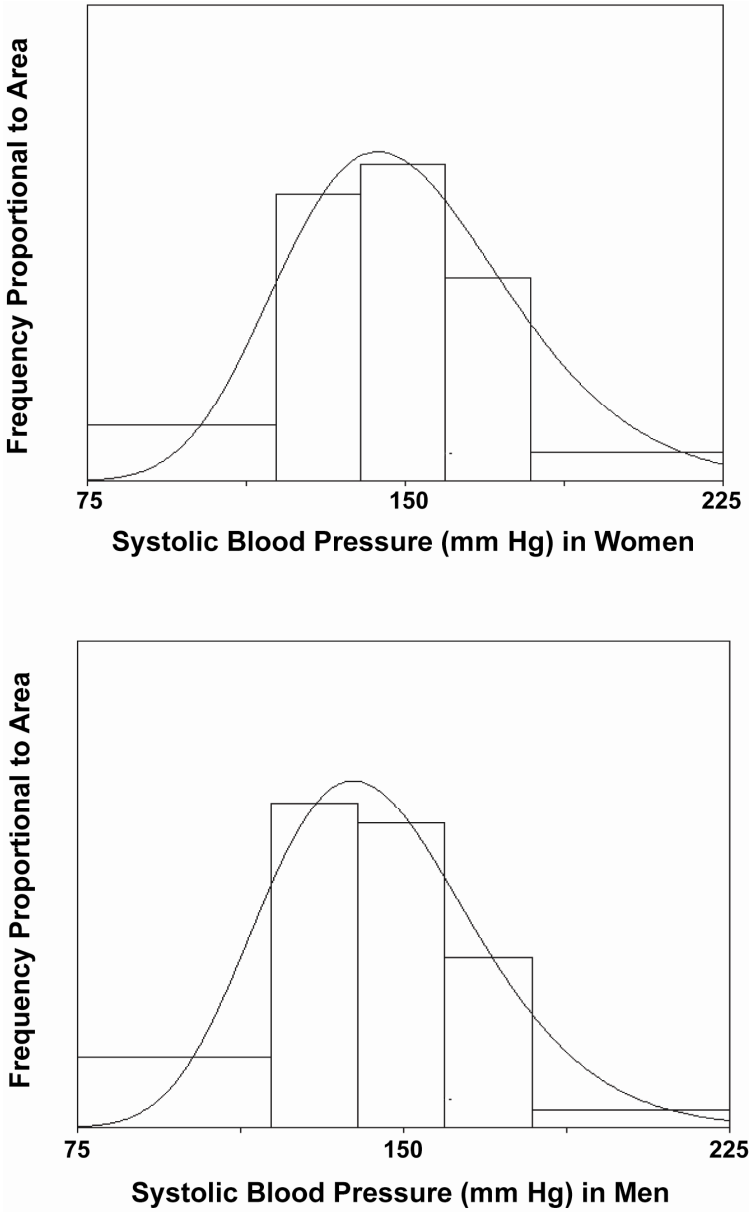


Figure 8-10. Distribution of systolic blood pressure in women and men aged 35 to 64 years from the Framingham Heart Study (Kannel, 2000a).

would translate to about 150 fewer events (e.g., heart attacks, strokes) for women and 240 fewer events for men, respectively. For a city of 10 million people (about the size of New York City) the estimated fewer serious cardiovascular events annually would be 500 and 800, respectively, for women and men.

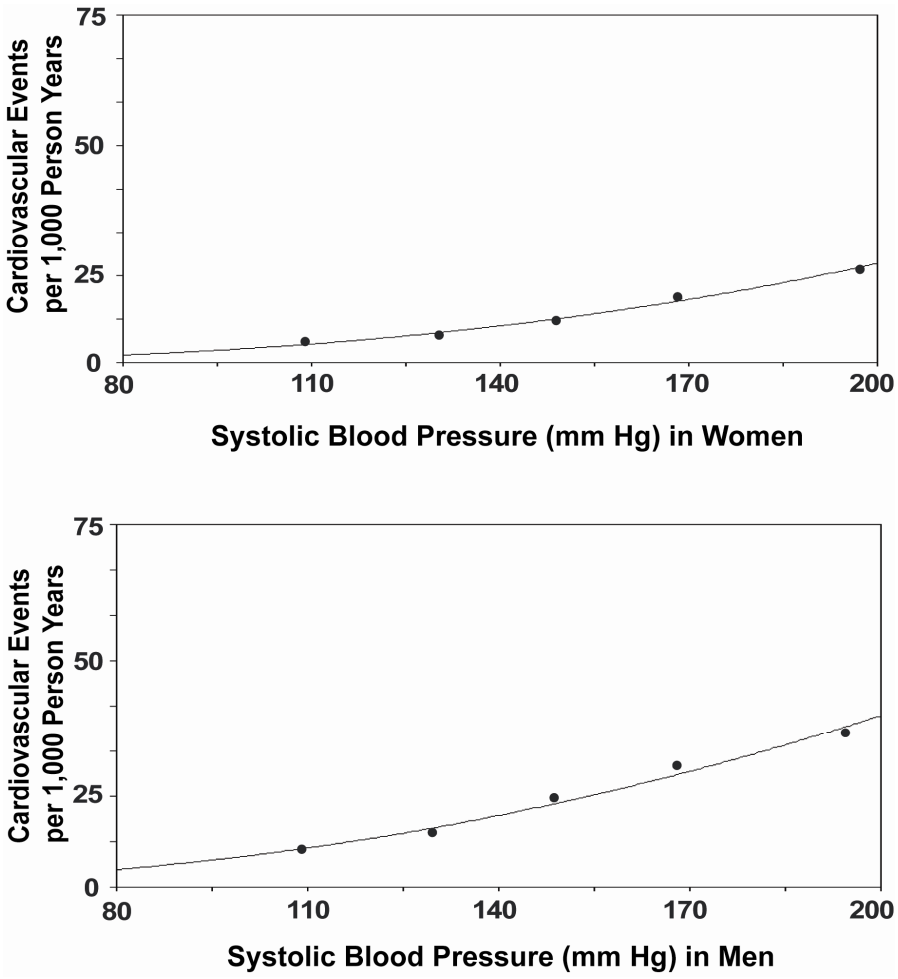


Figure 8-11. Relationship of serious cardiovascular events (coronary disease, stroke, peripheral artery disease, cardiac failure) to systolic blood pressure in women and men aged 35 to 64 years from the Framingham Heart Study (Kannel, 2000a).

Recent analyses of NHANES II and III data have yielded evidence supporting the likelihood that long-term Pb exposure can increase the risk of cardiovascular-related mortality in the general U.S. population, consistent with projected likely increases in serious cardiovascular events (stroke, heart attack) resulting from even small Pb-induced increases in blood pressure (as discussed above).

A recent follow-up of the NHANES II cohort provided mortality data used to associate past blood Pb concentration with increased circulatory mortality in the U.S. population (Lustberg

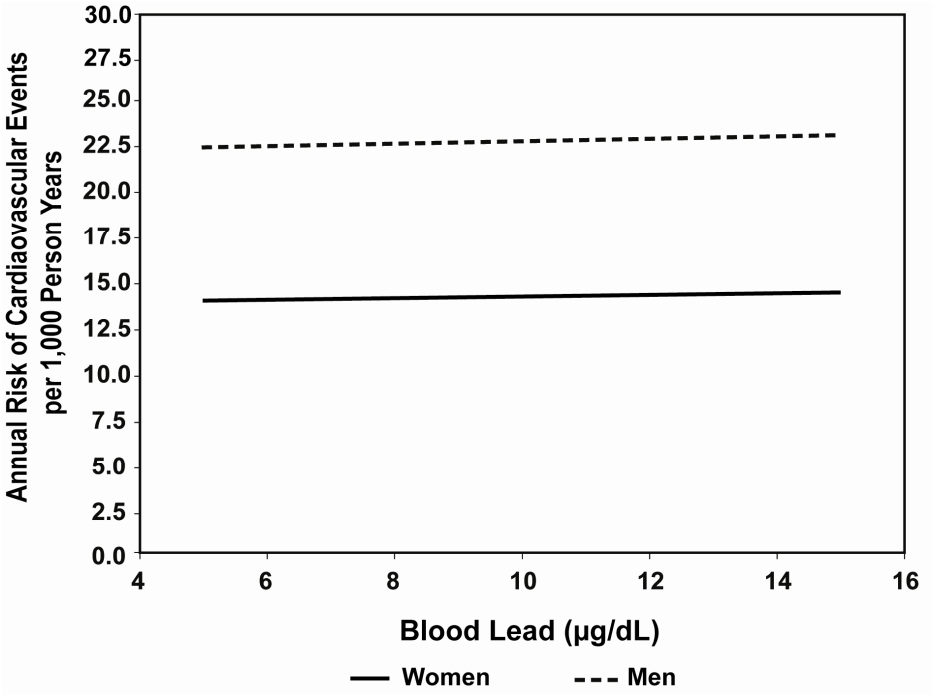


Figure 8-12. Effect of blood lead on expected annual risk of cardiovascular events per 1,000 person-years.

and Silbergeld, 2002). Blood Pb concentration as measured during 1976 to 1980 was divided into three categories (<10 µg/dL, 10-19 µg/dL, and 20-29 µg/dL) after eliminating 109 subjects with blood Pb ≥30 µg/dL, leaving 4,190 subjects 30-74 years of age in the mortality sample followed to the end of 1992. During the follow-up period, 929 subjects died of all causes. ICD-9 codes 390-459 (circulatory) accounted for 424 deaths. Proportional hazards models using a priori selected potential confounding variables (age, sex, race, education, income, smoking, BMI, exercise, and location) were used to calculate risk ratios of cardiovascular mortality for the two higher Pb categories compared to a <10 µg/dL reference. The 20-29 µg/dL category showed significantly elevated relative risk of 1.39 (95% CI: 1.01, 1.91) for cardiovascular mortality.

Although the NHANES II analysis using data from 1976 to 1980 suggested an increased risk of mortality at blood Pb levels above 20 µg/dL, blood Pb levels have dramatically decreased since the late 1970s. More recent data from NHANES have found that the geometric mean blood Pb levels decreased from 12.8 µg/dL in 1976-1980 to 2.8 µg/dL in 1988-1991 (Annest et al., 1983) and 2.3 µg/dL in 1991-1994 (CDC, 1997). NHANES III data (1988-1994) were

used to further analyze risk of mortality in adults (age 40 years) at lower blood Pb levels (Schober et al., 2006). A total of 9,757 subjects were followed for a median of 8.55 years during which there were 2,515 deaths. An increased risk of cardiovascular mortality was associated with blood Pb levels of 5-9 µg/dL and 10 µg/dL compared to 5 µg/dL. The relative risk was 1.20 [95% CI: 0.93, 1.55] for 5-9 µg/dL and 1.55 [95% CI: 1.16, 2.07] for 10 µg/dL, and the test for trend was statistically significant. Increased risks of all cause and cancer mortality also were observed at blood Pb levels of 5-9 µg/dL compared to <5 µg/dL (relative risk of 1.24 [95% CI: 1.05, 1.48] for all cause mortality and 1.44 [95% CI: 1.12, 1.86] for cancer mortality). The authors noted that an important limitation of this study was that exposure classification was based on one blood Pb level measurement taken at baseline (i.e., at 40 years old). These individuals were more likely to have notably higher past peak and cumulative Pb exposure, and their blood Pb levels might have been disproportionately influenced by release of Pb from bone stores compared to younger individuals.

The effects of Pb on serious cardiovascular events and mortality were projected to be more modest in two studies that estimated relative risks of serious cardiovascular outcomes from Pb-induced blood pressure changes using data from the NHANES II cohort. Pirkle et al. (1985) calculated that a 6.2 µg/dL increase in blood Pb levels (from 10.5 to 16.7 µg/dL) predicted a 4.6% increase in the incidence of fatal and nonfatal myocardial infarctions and 6.7% increase in the incidence of fatal and nonfatal strokes over 10 years in White men aged 40-54 years. The incidence of death from all causes was estimated to increase by 5.5% over 11.5 years with the same increase in blood Pb levels. Schwartz (1991) noted that doubling of blood Pb levels was associated with a relative risk of ~1.05 for cardiovascular disease in men and women aged 20-74 years.

Collectively, the above analyses of NHANES II and III data suggest a significant effect of Pb on cardiovascular mortality in the general U.S. population. Consideration of this health outcome may be qualitatively useful in helping to more fully understand potential public health impacts of Pb. However, the reasons for the notable differences between the above-noted analyses in estimated increased risk of cardiovascular-related fatal outcomes associated with incremental changes in blood Pb concentrations are unclear at this time. One likely explanation is the overestimation of risk by the Schober et al. (2006) analyses by relating ongoing mortality to relatively more recent baseline blood Pb levels in older adults than to past higher peak or

cumulative Pb exposures. Conversely, the Schober et al. estimates may, in part, reflect the contributions of Pb exposures to fatal cardiovascular outcomes mediated via other possible underlying mechanisms (e.g., Pb effects on heart rate variability, cholesterol metabolism, arthrosclerosis) in addition to Pb effects on blood pressure. Thus, until the Schober et al. findings are replicated and more fully understood, the Schober et al. (2006) estimates for Pb-induced cardiovascular mortality should probably not be used for quantitative risk assessment purposes.

8.6.4 Potential Implications of Renal Effects of Lead

The potential clinical relevance of Pb renal effects for chronic kidney disease has recently been examined. Chronic kidney disease is an important risk factor for cardiac disease and other causes of mortality and morbidity. Increasing blood lead from the 5th to the 95th percentile (3.5 µg/dL) has the same adverse impact on glomerular filtration as increases in age and body mass index (both known renal risk factors) among the general population. Further, a 10-fold increase in blood Pb (e.g., from 1 to 10 µg/dL) causes a 22.5% decrease in creatinine clearance in populations at high risk for Pb exposure. The biomedical significance of such altered creatinine clearance remains to be more fully elucidated, however, given observations in occupationally exposed groups of more notable renal dysfunction signs only at substantially higher blood-Pb levels (≤30-40 µg/dL).

A Pb-induced small downward shift in renal function among a general population may not alone result in chronic kidney disease in identifiable individuals; however, the segment of the population with the lowest renal reserve may be put at increased risk for chronic kidney disease when Pb exposure is combined with one or more other renal risk factors. Effect estimates in susceptible populations, such as those with diabetes, hypertension, or chronic renal insufficiency from non-Pb related causes, are likely to be higher. Lead exposure in populations that are also at increased risk for obesity, diabetes, and hypertension represent groups likely to be the most impacted by Pb. Frequently both risk factors are present in the same lower socioeconomic status groups.

8.6.5 Potential Implications of Lead-Induced Immune System Effects

Disease implications associated with Pb-induced immune changes seen in animals are likely to include an increased risk of allergic diseases, atopic manifestations and possibly later-life autoimmunity as well as a reduced capacity to combat certain viral infections and cancers. Diseases associated with hyperinflammation would also be of concern. A recent mechanistic study in the mouse produced two major findings (see Section 5.9.8): (1) it confirmed the capacity of Pb to induce a Th2 bias, increasing allergic disease concerns; and (2) it showed that Pb exposure elevates immune reaction against neoantigens, thereby increasing the risk of autoimmune reactions.

8.7 KEY LEAD ECOSYSTEM EFFECTS AND POTENTIAL IMPLICATIONS

8.7.1 Terrestrial Ecosystems

Surface soils across the United States are enriched in lead (Pb) relative to levels expected from natural (geogenic) inputs. While some of this Pb contamination is attributable to paint, salvage yards, shooting ranges, and the use of Pb arsenate as a pesticide in localized areas, Pb contamination of surface soils is essentially ubiquitous because of atmospheric pollution associated with past widespread use of leaded gasoline and more contemporary metal smelting and production, combustion of fossil fuels, and waste incineration (see Table 2-8). However, lead inputs to terrestrial ecosystems in the United States have declined dramatically in the past 30 years, due to the almost complete elimination of alkyl-lead additives in gasoline in North America. Also, emissions from smelters have declined as older plants have been shut down or fitted with improved emissions controls.

Most terrestrial ecosystems in North America remain sinks for Pb, despite reductions in atmospheric Pb deposition of more than 95% during the past several decades. Lead released from forest floor soils in the past has been largely immobilized in mineral soils (see Section 8.1). The amount of Pb that has leached into the mineral soil to date has been estimated to range from 20 to 90% of past total anthropogenic Pb deposition, depending on forest type, climate, and litter cycling. While inputs of Pb to ecosystems are currently low, Pb export from watersheds via groundwater and streams appears to be substantially lower, Pb concentrations in waters draining natural terrestrial ecosystems always having been reported as low (generally less than 1 ng/L),

even at moderately polluted sites. Therefore, even at current low input levels, U.S. watersheds are accumulating industrial Pb. However, burial/movement of lead over time down into lower soil/sediment layers also tends to sequester it away from more biologically active parts of the watershed (unless later disturbed or redistributed, e.g., by flooding, dredging, etc.).

Metal Speciation for Plants

When considering the bioavailability of a metal to plants from soils and sediments, it is generally assumed that both the kinetic rate of supply and the speciation of the metal to either the root or shoot are highly important. In soils and sediments, generally only a small volume of water is in contact with the chemical form; and, although the proportion of the concentration of a metal in this pore water to the bulk soil/sediment concentration is small, it is this phase that is directly available to plants. Therefore, pore water chemistry, i.e., metal concentration as simple inorganic species, organic complexes, or colloid complexes, is most important. Tools currently used for metal speciation for plants include (1) in situ measurements using selective electrodes; (2) in situ collection techniques using diffusive equilibrium thin films (DET) and diffusive gradient thin films (DGT) followed by laboratory analyses; and (3) equilibrium models (e.g., SOILCHEM) (see Section 7.1.1 and AX7.1.1.2).

Lead Speciation in Solid Phases

Lead can enter terrestrial ecosystems through natural rock weathering and by a variety of anthropogenic pathways. During the hydrolysis and oxidation of Pb-containing minerals, divalent Pb (Pb^{2+}) is released to the soil solution, where it is rapidly fixed by organic matter and secondary mineral phases. The geochemical form of natural Pb in terrestrial ecosystems is strongly controlled by soil type and parent material (see Annex Section AX7.1.2.1). In contrast, anthropogenically-introduced Pb has a variety of different geochemical forms, depending on the specific source. While Pb in soils from battery reclamation areas can be in the form of $PbSO_4$ or $PbSiO_3$, Pb in soils from shooting ranges and paint spills is commonly found as PbO and a variety of Pb carbonates. Atmospherically-delivered Pb from fossil fuel combustion is typically introduced into terrestrial ecosystems as Pb-sulfur compounds and Pb oxides. After deposition, most Pb species are likely transformed. Although the specific factors that control the speciation of anthropogenic Pb in soils are not well understood, there are many studies that have partitioned

Pb into its different geochemical phases. In most cases, Pb appears to be strongly bound to soils and sediments in terrestrial ecosystems, which prevents substantial mobility and uptake in the terrestrial environment. However, the controls on Pb speciation, and thus on mobility and potential bioavailability are not completely understood, so there remains a considerable need for more research on this topic. A thorough understanding of Pb speciation is very important in order to predict potential mobility and bioavailability and in order to accurately apply a critical loads methodology for determining air quality standards (see Section 7.3).

Selective chemical extractions have been employed extensively for quantifying amounts of a particular metal phase present in soil rather than total metal concentration. However, some problems persist with the selective extraction technique. First, extractions are rarely specific to a single phase. In addition to non-selectivity of reagents, significant metal redistribution has been documented during sequential chemical extractions, and many reagents may not extract targeted phases completely. Therefore, while chemical extractions do provide some useful information on metal phases in soil and scenarios for mobilization, the results should be treated as "operationally defined," e.g., "H_2O_2 liberated-Pb" rather than "organic Pb."

Selective chemical extractions and synchrotron-based X-ray studies have shown that industrial Pb can be strongly sequestered by organic matter and secondary minerals such as clays and oxides of Al, Fe, and Mn. More recent X-ray studies have further demonstrated the importance of biomineralization of Pb in soils by bacteria and nematodes.

Lead Solid-solution Partitioning

The concentration of Pb species dissolved in soil solution is probably controlled by some combination of (a) Pb mineral solubility equilibria; (b) adsorption reactions of dissolved Pb phases on inorganic surfaces (e.g., oxides of Al, Fe, Si, Mn, etc., clay minerals); and (c) adsorption reactions of dissolved Pb phases on soil organic matter. Dissolved Pb phases in soil solution can be some combination of Pb^{2+} and its hydrolysis species, Pb bound to dissolved organic matter, and Pb complexes with inorganic ligands such as Cl^- and SO_4^{2-}. Alkaline soils typically have solutions supersaturated with respect to $PbCO_3$, $Pb_3(CO_3)_2(OH)_2$, $Pb(OH)_2$, $Pb_3(PO_4)_2$, $Pb_5(PO_4)_3(OH)$, and $Pb_4O(PO_4)_2$. Pb phosphate minerals in particular, are very insoluble, and calculations based on thermodynamic data predict that these phases will control dissolved Pb in soil solution under a variety of conditions. However, certain chelating agents,

such as dissolved organic matter can prevent the precipitation of Pb minerals and the natural formation of these minerals has not yet been observed in terrestrial ecosystems (see AX7.1.2.1).

Soil solution dissolved organic matter content and pH typically have a very strong positive and negative correlation, respectively, with the concentration of dissolved Pb species. In the case of adsorption phenomena, the partitioning of Pb^{2+} to the solid phase is also controlled by total metal loading: high Pb loadings will result in a lower fraction partitioned to the solid phase. It has been found that only a fraction of the total Pb in solution was actually Pb^{2+} in soils treated with leaf compost. The fraction of Pb^{2+} to total dissolved Pb ranged from <1 to 60%, depending on pH and the availability of Pb-binding ligands. In acidic soils, Al species can compete for sites on natural organic matter and inhibit Pb binding to surfaces.

Tracing the Fate of Atmospherically Delivered Lead

Radiogenic Pb isotopes offer a powerful tool for separating anthropogenic Pb from natural Pb derived from mineral weathering (see AX7.1.2.2). This is particularly useful for studying Pb in mineral soil, where geogenic Pb often dominates. The ore bodies from which anthropogenic Pb are typically derived are usually enriched in ^{207}Pb relative to ^{206}Pb and ^{208}Pb when compared with Pb found in granite rocks. Uranium-238 series ^{210}Pb also provides a tool for tracing atmospherically delivered Pb in soils. Fallout ^{210}Pb is deposited onto forests via wet and dry deposition, similar to anthropogenic Pb deposition in forests and is thusly useful as a tracer for non-native Pb in soils. ^{210}Pb is convenient to use for calculating the residence time of Pb in soil layers because its atmospheric and soil fluxes can be assumed to be in steady-state at undisturbed sites.

Researchers assessing the fate of atmospheric Pb in soils have also relied on repeated sampling of soils and vegetation for total Pb. This technique works best when anthropogenic Pb accounts for the vast majority of total Pb in a particular reservoir. Evans et al. (2005), for example, have noted that surface soils sampled relatively recently demonstrate that the upper soil horizons (O + A horizons) have been retaining most of the anthropogenic Pb burden introduced to the systems during the 20th century, and others have suggested that lateral and vertical movement of organic particles dominated Pb transport in the soil profile (see AX7.1.2.2).

By describing the movement of atmospherically-delivered Pb in terrestrial ecosystems, we can begin to predict the Pb inventories of various ecosystem compartments that a particular

atmospheric deposition rate will support. This type of information is very pertinent to air quality issues. For example, if the rate of Pb loss is known for a particular soil horizon and reasonable assumptions can be made about biogeochemical cycling and chemical weathering inputs, then steady-state Pb concentrations can be calculated for any constant deposition rate (in mass of Pb deposited per square meter). First-order rate loss constants, k, have been calculated for organic horizons using forest floor inventories, radiogenic ^{207}Pb tracer techniques, and fallout ^{210}Pb. First order rate loss constants vary substantially, ranging between −0.003 to −0.6 (1/y), depending on soil type and climate. First-order rate loss techniques used to model forest floor Pb dynamics at the Hubbard Brook Experimental Forest in New Hampshire revealed that with steady Pb deposition at 0.0065 kg/ha/y, the forest floor would reach a steady-state Pb concentration of 1.4 ppm. Calculated steady-state Pb contents of different ecosystem compartments can then be compared with experimentally-derived toxicity thresholds to put deposition rates into context with the terrestrial ecosystem.

Uptake into Plants and Invertebrates

Recent work supports previous conclusions that the form of metal tested, and its speciation in soil, influence uptake and toxicity to plants and invertebrates. The oxide Pb form is less toxic than the chloride or acetate forms, which are less toxic than the nitrate form of Pb. However, these results must be interpreted with caution, as the counterion (e.g., the nitrate ion) may be contributing to the observed toxicity (see AX7.1.3.1). Most Pb is taken up by plants via the symplastic route (through cell membranes) and remains in the roots, with little translocation to shoots, leaves, or other plant parts. Different species of plants and invertebrates accumulate different amounts of lead.

Detoxification in Plants and Invertebrates

Lead may be deposited in root cell walls as a detoxification mechanism, and this may be regulated by calcium precipitates in the cell wall. The oxalate content in root and root exudates may reduce the bioavailability of Pb in soil, and constitute an important tolerance mechanism. Other hypotheses put forward recently include (a) the presence of sulfur ligands and (b) the sequestration of Pb in old leaves as detoxification mechanisms. Lead detoxification has not been studied extensively in invertebrates. Glutathione detoxification enzymes were measured in two

species of spider; and Pb may be stored in waste nodules in earthworms or as pyromorphite in nematodes.

Physiological Effects

The effects on heme synthesis, as measured by 5-aminolaevulinic acid dehydratase (ALAD) activity and protoporphyrin concentration, primarily, were well-documented in the 1986 AQCD (U.S. Environmental Protection Agency, 1986) and continue to be studied in birds and mammals. However, Henny et al. (1991) caution that changes in ALAD and other enzyme parameters are not always related to adverse effects, but may simply indicate exposure. Other effects on plasma enzymes that may damage other organs have been reported (see AX7.1.3.3). Lead also may cause lipid peroxidation which may be alleviated by Vitamin E, although Pb poisoning may still result. Also, changes in fatty acid production have been reported, which may influence immune response and bone formation.

Response Modification

Genetics, biological factors, physical/environmental factors, nutritional factors, and interactions with other pollutants can all modify terrestrial organism response to Pb (see AX7.1.3.4). Some species are more sensitive to Pb than others. For example, Fisher 344 rats were found to be more sensitive to Pb than Sprague-Dawley rats. Also, younger animals are more sensitive than older animals, and females generally more so than males. Too, monogastric animals are more sensitive than ruminants, insectivorous mammals may be more exposed to Pb than herbivores, and higher tropic-level consumers may be less exposed than lower trophic-level organisms. Diets deficient in nutrients (including low calcium) result in increased uptake of Pb and greater toxicity in birds, relative to diets containing adequate nutrient levels. Data on effects of Pb interactions with other metals vary, depending on the endpoint measured, the tissue analyzed, the animal species, and the metal combination.

Mycorrhizal fungi may ameliorate Pb toxicity until a threshold is surpassed, which may explain why some studies show increased uptake into plants while others show no difference or less uptake. Uptake of lead into plants and soil invertebrates increases with a decrease in soil pH. However, calcium content, organic matter content, and cation exchange capacity of soils also can significantly influence Pb uptake into plants and invertebrates (see AX7.1.3.4).

Primary Producers

Effects of lead on terrestrial plants include decreased photosynthetic and transpiration rates, and decreased growth and yield. The phytotoxicity of lead is considered to be relatively low compared to other metals, and there are few reports of phytotoxicity from Pb exposure under field conditions. Data on phytotoxicity were recently reviewed for development of ecological soil screening levels (Eco-SSL) (U.S. Environmental Protection Agency, 2005b). Many of the toxicity data presented in U.S. Environmental Protection Agency (2005b) are lower (i.e., they represent greater toxicity) than those discussed in the 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986), although both documents acknowledge that toxicity is observed over a wide range of concentrations of Pb in soil (tens to thousands of mg/kg soil). This may be due to many factors, such as the soil conditions (e.g., pH, organic matter) and differences in bioavailability of the Pb in spiked soils, perhaps due to lack of equilibration of the Pb solution with the soil after spiking. Most phytotoxicity data continue to be developed for agricultural plant species (i.e., vegetable and grain crops). Few data are available for trees or native herbaceous plants, although two of the five ecotoxicological endpoints used to develop the Eco-SSL were for trees and two were for clover.

Consumers

Lead effects on avian and mammalian consumers include decreased reproduction, growth, and survival, as well as effects on development and behavior. Only relatively few field effects data exist for consumers, except from sites with multiple contaminants, for which it is difficult to attribute toxicity specifically to Pb. Much of the avian and mammalian toxicity data recently reviewed for the development of Eco-SSLs (U.S. Environmental Protection Agency, 2005b) are lower than those discussed in the 1986 Lead AQCD (i.e., the Eco-SSL document describes studies which report greater toxicity of Pb to various organisms) although EPA (U.S. Environmental Protection Agency, 2005b) recognizes that toxicity is observed over a wide range of doses (<1 to >1,000 mg Pb/kg bw-day). Most toxicity data for birds are derived from chicken and quail studies, and most data for mammals are derived from laboratory rat and mouse studies. Data derived for other species would contribute to increased understanding of Pb toxicity, particularly for wildlife species with different gut physiologies. In addition, data derived using environmentally-realistic exposures, such as from Pb-contaminated soil and food may be

recommended. Finally, data derived from inhalation exposures that evaluate endpoints such as survival, growth, and reproduction would enhance understanding the implications of airborne releases of Pb.

Decomposers

Lead effects on soil invertebrates include decreased survival, growth and reproduction. Effects on microorganisms include changes in nitrogen mineralization and in enzyme activities. Recent data on Pb toxicity to soil invertebrates and microorganisms are consistent with those reported in the 1986 Lead AQCD, with toxicity generally observed at concentrations of 100's to 1,000's of mg Pb/kg soil. Studies on microbial processes may be influenced significantly by soil parameters, and the significance of the test results is not clear.

Ecological Soil Screening Levels (Eco-SSLs)

Eco-SSLs are concentrations of contaminants in soils that would result in little or no measurable effect on ecological receptors. They were developed by U.S. EPA for use in the screening level assessments at Superfund sites to identify those contaminants needing further investigation, and also to identify those contaminants that are not of potential ecological concern and do not need to be considered in the subsequent analyses. However, several conservative factors were incorporated into their development. For the plant and invertebrate Eco-SSLs, studies were scored to favor relatively high bioavailability. For wildlife Eco-SSLs, only species with a clear exposure link to soil were considered (generalist species, species with a link to the aquatic environment, or species which consume aerial insects were excluded), simple diet classifications were used (100% plants, 100% earthworms or 100% animal prey) when in reality wildlife consume a varied diet, species were assumed to forage exclusively at the contaminated site, relative bioavailability or Pb in soil and diet was assumed to be 1, and the TRV was selected as the geometric mean of NOAELs unless this value was higher than the lowest bounded LOAEL for mortality, growth or reproduction. The Eco-SSLs are intentionally conservative in order to provide confidence that contaminants which could present an unacceptable risk are not screened out early in the evaluation process. That is, at or below these levels, adverse effects are considered unlikely. Due to conservative modeling assumptions (e.g., metal exists in most toxic form or highly bioavailable form, high food ingestion rate, high soil ingestion rate) which are

common to screening processes, several Eco-SSLs are derived below the average background soil concentration for a particular contaminant. The Pb Eco-SSLs for terrestrial plants, birds, mammals, and soil invertebrates are 120 mg/kg, 11 mg/kg, 56 mg/kg, and 1700 mg/kg, respectively. (For additional information see Annex Section AX7.1.4).

Effects of Lead on Natural Terrestrial Ecosystems

Few significant effects of Pb pollution have been observed at sites that are not near point sources of Pb. At present, industrial point sources such as smelter sites represent the greatest Pb-related threat to the maintenance of sustainable, healthy, diverse, and high-functioning terrestrial ecosystems in the United States. However, assessing the risks specifically associated with Pb is difficult because these sites also experience elevated concentrations of other metals and because of effects related to SO_2 emissions. Terrestrial ecosystems may respond to stress in a variety of ways, including reductions in the vigor and/or growth of vegetation, reductions in biodiversity, and effects on energy flow and biogeochemical cycling.

Influence of Acidification

Like most metals, the solubility of Pb increases as pH decreases, suggesting that enhanced mobility of Pb should be found in ecosystems under acidification stress. However, Pb is also strongly bound to organic matter in soils and sediments. Reductions in pH may cause a decrease in the solubility of dissolved organic matter (DOM), due to the protonation of carboxylic functional groups. Because of the importance of Pb complexation with organic matter, lower DOM concentrations in soil solution resulting from acidification may offset the increased solubility of Pb and hence decrease the mobility of the organically bound metal. Increased mobility was only observed in very acidic soils, those with pH <4.5 (see AX7.1.5.1). Acidification also may enhance Pb export to drainage water in very sandy soils that have limited ability to retain organic matter.

Influence of Land Use and Industry

Changes in land use represent potentially significant changes in the cycling of organic matter in terrestrial ecosystems. Conversion of pasture and croplands to woodlands changes the nature and quantity of organic matter inputs to the soil. The introduction of industrial activity

may have consequences for organic matter cycling, and subsequently, Pb mobilization. In one rare long-term study of polluted soils, loss of soil carbon was found to induce the mobilization and loss of Pb from terrestrial ecosystems. However, it is worth noting that the decline in soil Pb was considerably smaller than the decline in organic carbon. This suggests that Pb mobilized during organic matter decomposition can resorb to remaining organic matter or perhaps to alternate binding sites (e.g., Fe and Mn oxides).

Forest harvesting represents a severe disruption of the organic matter cycle in forest ecosystems. However, observations from clear-cut sites in the United States and Europe indicate that forest harvesting causes little or no mobilization or loss of Pb from forest soils. The principal risk associated with forest harvesting is the loss of Pb in particulate form to drainage waters through erosion.

Effects Observed Around Industrial Point Sources

The effects of Pb exposure on natural ecosystems are confounded by the fact that Pb exposure cannot be decoupled from other factors that may also affect the ecosystem under consideration. Principal among these factors are other trace metals and acidic deposition. Emissions of Pb from smelting and other industrial activities are accompanied by other trace metals (e.g., Zn, Cu, Cd) and sulfur dioxide (SO_2) that may cause toxic effects independently or in concert with Pb.

Natural terrestrial ecosystems near smelters, mines, and other industrial plants have exhibited a variety of effects related to ecosystem structure and function. These effects include decreases in species diversity, changes in floral and faunal community composition, and decreasing vigor of terrestrial vegetation (see AX7.1.5.2). Subsequent to the effects on vegetation, wind and erosion may remove litter and humus, leaving bare mineral soil, a nearly sterile environment in which very little energy transfer takes place. In a rare case, metal pollution around a Pb-Zn smelter near Bristol, England has not resulted in the loss of oak woodlands within 3 km of the smelter, despite significant accumulation of Pb, Cd, Cu, and Zn in soils and vegetation. However, the high metal concentrations have favored the growth of metal-tolerant species in the woodland (see AX7.1.5.2). The effects of Pb and other chemical emissions on terrestrial ecosystems near smelters and other industrial sites decrease downwind from the Pb source. Several studies using the soil Pb burden as an indicator have shown that

much of the contamination occurs within a radius of 20 to 50 km around the emission source. Elevated metal concentrations around smelters have been found to persist despite significant reductions in emissions. The confounding effect of other pollutants makes the assessment of Pb-specific exposure-response relationships impossible at the whole-ecosystem level.

Influence of Climate Change

Atmospheric Pb is not likely to contribute significantly to global climate change. The potential linkages between climate-related stress and Pb cycling are poorly understood. Climate effects related to alterations in organic matter cycling may influence Pb migration. For example, an increase in temperature leading to increased rates of organic matter decomposition could lead to temporary increases in DOM concentrations and smaller steady-state pools of soil organic matter. There also is some evidence for recent increases in the frequency of soil freezing events in the northeastern United States. Soil freezing occurs when soils have little or no snow cover to insulate them from cold temperatures and results in an increased release of nitrate and DOC from the O horizons of forest soils. Increased fluctuations in precipitation may induce more frequent flooding, potentially increasing inputs of Pb and other metals to floodplain soils. All of these factors could result in increased concentrations of Pb in waters draining terrestrial ecosystems.

Influence on Energy Flow and Biogeochemical Cycling

Lead can have a significant effect on energy flow in terrestrial ecosystems. In terrestrial ecosystems, energy flow is closely linked to the carbon cycle. The principal input of energy to terrestrial ecosystems is through photosynthesis, in which CO_2 is converted to biomass carbon. Because of this link between photosynthesis and energy flow, any effect that Pb has on the structure and function of terrestrial ecosystems influences the flow of energy into the ecosystem. At some sites severely affected by metal pollution, death of vegetation can occur, dramatically reducing the input of carbon to the ecosystem (see AX7.1.5.3).

Lead influences energy transfer within terrestrial ecosystems, which begins with the decomposition of litter and other detrital material by soil bacteria and fungi, and cascades through the various components of the detrital food web. In acid- and metal-contaminated soils or soils treated with Pb, investigators have documented significant declines in litter decomposition rates and/or the rate of carbon respiration in acid- and metal-contaminated soils or

soils treated with Pb (see AX7.1.5.3). The resulting accumulation of organic matter on the soil surface can be dramatic.

Because the mobility of Pb in soils is closely tied to organic matter cycling, decomposition processes are central to the biogeochemical cycle of Pb. Reduced decomposition rates in polluted ecosystems are the result of the inhibition of soil bacteria and fungi and its effects on microbial community structure (see AX7.1.5.3). Lead and other metals also inhibit the mineralization of nitrogen from soil organic matter and nitrification, resulting in lower nitrogen availability to plants. This suggests that the inhibitory effect of Pb and other metals is broad-based, and not specific to any particular metabolic pathway. It is important to note that terrestrial sites that have exhibited significant disruption to energy flows and C processing are sites that have experienced severe metal contamination from smelters or other metals-related activities.

8.7.2 Aquatic Ecosystems

Sediment Quality Benchmarks and Bioavailability

There are a number of factors in sediment that can influence lead bioavailability to benthic (sediment) organisms. Although sediment quality criteria have not been formally adopted, the EPA has published an equilibrium partitioning procedure for developing sediment criteria for metals. Equilibrium partitioning (EqP) theory predicts that metals partition in sediment between acid volatile sulfide, pore water, benthic organisms, and other sediment phases, such as organic carbon. Using this theory, sediment toxicity and organism mortality can be more reliably predicted by accounting for both the site-specific organic carbon and AVS concentrations. It should be noted that although EPA is favoring the AVS-SEM approach for regulating metals in sediments, there is not scientific consensus on this issue. Various studies suggest that ingestion of sediment particles by benthic organisms is an important exposure route not accounted for by AVS-SEM or that the AVS-SEM approach may not be the most accurate approach available for predicting non-toxic and toxic results in laboratory studies.

Speciation of Lead in Aquatic Ecosystems

The speciation of Pb in the aquatic environment is controlled by many factors, such as pH, salinity, sorption, and biotransformation processes. Lead is typically present in acidic aquatic environments as $PbSO_4$, $PbCl_4$, ionic Pb, cationic forms of Pb hydroxide, and ordinary hydroxide

$Pb(OH)_2$. In alkaline, waters common Pb species include anionic forms of Pb carbonate $Pb(CO_3)$ and hydroxide $Pb(OH)_2$. In freshwaters, Pb typically forms strong complexes with inorganic OH^- and CO_3^{2-} and weak complexes with Cl^-. The primary form of Pb in freshwaters at low pH ($\leq$6.5) is predominantly Pb^{2+}; and less abundant inorganic forms include $Pb(HCO)_3$, $Pb(SO_4)_2^{2-}$, PbCl, $PbCO_3$, and $Pb_2(OH)_2CO_3$. At higher pH ($\geq$7.5) Pb forms hydroxide complexes ($PbOH^+$, $Pb(OH)_2$, $Pb(OH)_3^-$, $Pb(OH)_4^{2-}$). Lead speciation in seawater is a function of chloride concentration and the primary species are $PbCl^{3-}$ > $PbCO_3$ > $PbCl_2$ > $PbCl^+$ > and $Pb(OH)^+$ (see AX7.2.2.1).

Lead sorption to suspended or bed sediments or suspended organic matter typically increases with increasing pH, increasing amounts of iron or manganese; and with the polarity of component particulate matter (e.g., clays). Adsorption decreases with water hardness. At higher pH, Pb precipitates as $Pb(OH)^+$ and $PbHCO_3^+$ into bed sediments. Conversely, at low pH, Pb is negatively sorbed, i.e., repelled from the adsorbent surface (see AX7.2.2.1). Also, Pb may be remobilized from sediment due to a decrease in metal concentration in the solution phase, complexation with chelating agents (e.g., EDTA), and changing redox conditions. Changes in water chemistry (e.g., reduced pH or ionic composition) can cause sediment Pb to become re-mobilized and potentially bioavailable to aquatic organisms. Methylation may result in Pb remobilization, its reintroduction into the aqueous environmental compartment, and its subsequent release into the atmosphere. However, methylation is not a significant environmental pathway controlling Pb fate in the aquatic environment.

Lead Concentrations in United States Surface Waters

Nationwide U.S. data for Pb in surface waters, from 1991 onward, were compiled using the United States Geological Survey's (USGS) National Water-Quality Assessment (NAWQA) database. Data were compiled from locations categorized as "ambient" or "natural." Ambient refers to data collected from all sampling locations, while natural refers to data collected from sampling locations categorized as forest, rangeland, or reference. Summary statistics for surface water, sediment (bulk, <63 um), and fish tissue (whole body and liver) are summarized in Table 8-8. Overall, atmospheric sources of Pb have generally decreased as regulations have removed Pb from gasoline and other products; however, elevated Pb concentrations remain at sites near ongoing sources, such as near mining wastes or wastewater effluents.

Table 8-8. Summary of Lead Concentrations in United States Surface Water, Sediment, and Fish Tissue

	Surface Water – Dissolved (µg/L)		Sediment – Bulk, <63 µm (µg/g dry wt.)		Fish Tissue (µg/g dry wt.)			
					Whole Organism		Liver	
Statistic	**Ambient**	**Natural**	**Ambient**	**Natural**	**Ambient**	**Natural**	**Ambient**	**Natural**
n	3,445	430	1,466	258	332	93	559	83
%ND	86	88	0.48	1.2	39	51	71	89
Min	0.04	0.04	0.50	0.50	0.08	0.08	0.01	0.01
Mean	0.66	0.52	120	109	1.03	0.95	0.36	0.28
Median	0.50	0.50	28	22	0.59	0.35	0.15	0.11
90th %ile	0.50	0.50	120	66	2.27	1.40	0.59	0.37
95th %ile	1.10	0.50	200	162	3.24	2.50	1.06	1.26
Max	29.78	8.40	12,000	12,000	22.6	22.6	12.7	3.37

%ND = Percentage not detected

Lead concentrations in lakes and oceans were generally found to be much lower than those measured in the lotic waters assessed by NAWQA. Surface water concentrations of dissolved Pb measured in Hall Lake, Washington in 1990 ranged from 2.1 to 1015.3 ng/L, and the average surface water dissolved Pb concentrations measured in the Great Lakes (Superior, Erie, and Ontario) between 1991 and 1993 were 3.2, 6.0, and 9.9 ng/L, respectively. Lead concentrations ranged from 3.2 to 11 ng/L across all three lakes. Similarly, 101 surface water total Pb concentrations measured at the Hawaii Ocean Time-series (HOT) station ALOHA between 1998 and 2002 ranged from 5 to 11 ng/kg. Based on the fact that Pb is predominately found in the dissolved form in the open ocean (<90%), dissolved Pb concentrations measured at these locations would likely have been even lower than the total Pb concentrations reported.

In addition to directly measuring Pb concentrations in various aquatic compartments, it is useful to study the vertical distribution of Pb. Sediment profiling and core dating is a method used to determine the extent of accumulation of atmospheric Pb and provides information on potential anthropogenic sources. Sediment concentration profiles are typically coupled with lead isotopic analysis. The isotope fingerprinting method utilizes measurements of the abundance of common lead isotopes (^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb) to distinguish between natural Pb over geologic

time and potential anthropogenic sources. Studies of sediment profiles have suggested that observed increases in Pb concentrations in the upper sediment layer are concomitant with increases in anthropogenic inputs. Isotopic ratios have been used to link increases in sediment concentrations with specific anthropogenic sources and to estimate historic records of Pb fluxes to surface waters and sediments (see AX7.2.2.3).

Lead Uptake

Lead can bioaccumulate in the tissues of aquatic organisms through ingestion of food and water, and adsorption from water, and can subsequently lead to adverse effects if tissue concentrations are sufficiently high. The accumulation of Pb is influenced by pH and decreasing pH favors bioavailability and bioaccumulation. Organisms that bioaccumulate Pb with little excretion must partition the metal such that it has limited bioavailability, otherwise toxicity will occur if a sufficiently high concentration is reached (see AX7.2.3.1).

Resistance Mechanisms

Aquatic organisms have various methods to resist the toxic effects of metals such as Pb. Mechanisms of resistance vary among aquatic biota and may include detoxification and avoidance responses. Detoxification processes can include translocation, excretion, chelation, adsorption, and vacuolar storage and deposition. For example, protists and plants produce intracellular polypeptides that form complexes with Pb. Some macrophytes and wetland plants have developed translocation strategies for tolerance and detoxification. Various aquatic invertebrates may sequester Pb in the exoskeleton or have developed specialized excretion processes. Fish scales and mucous may chelate Pb in the water column and potentially reduce Pb uptake (see AX7.2.3.2).

Avoidance responses are actions performed to evade a perceived threat. Some aquatic organisms have been shown to be quite adept at avoiding Pb in aquatic systems, while others seem incapable of detecting its presence. Snails have been shown to be sensitive to Pb, and avoid it at high concentrations. Conversely, anuran (frog and toad) species lack an avoidance response up to 1000 µg Pb/L. Fish avoidance of chemical toxicants has been well established and is a dominant sublethal response in polluted waters. However, studies examining avoidance behavior of Pb in fish are lacking (see AX7.2.3.2).

Physiological Effects of Lead

Physiological effects of Pb on aquatic biota can occur at the biochemical, cellular and tissue levels of organization. Lead has been shown to affect brain receptors in fish and serum enzyme activity (e.g., EROD and ALAD) in fish and amphibians. Studies examining Pb effects on fish blood chemistry have indicated alterations from acute and chronic exposures ranging from 100 to 10,000 µg/L. Lead exposure has also been shown to negatively affect the growth of aquatic invertebrates (see AX7.2.3.3).

Factors that Modify Organism Response to Lead

There are several factors that may modify responses of aquatic organisms to Pb exposure. These may include the size or age of an organism, genetics, environmental factors (e.g., pH, salinity), nutrition, and the presence of other contaminants. Lead accumulation in living organisms is controlled, in part, by metabolic rates and by the physiological conditions of an organism. Relationships between age, size and Pb body burden in aquatic invertebrates and fish are variable and depend on many environmental variables (e.g., exposure). For example, examination of Pb exposure (up to 100 µg/L) in aquatic invertebrates showed little relationship between body size and Pb accumulation (MacLean et al., 1996; Canli and Furness, 1993) while Pb accumulation and fish size were positively correlated (Douben, 1989; Köck et al., 1996).

The genetics of an organism and/or population may alter the response to Pb exposure through one of two processes: (1) a contaminant may influence selection, by selecting for certain phenotypes that enable populations to better cope with the chemical, or (2) a contaminant can be genotoxic, meaning it can produce alterations in nucleic acids at sublethal exposure concentrations, resulting in changes in hereditary characteristics or DNA inactivation. Genetic selection has been observed in aquatic organisms due to lead tolerance. Because tolerant individuals have a selective advantage over vulnerable individuals in polluted environments, the frequency of tolerance genes will increase in exposed populations over time. Several studies have shown that heavy metals can alter population gene pools resulting in decreased genetic diversity. Laboratory studies have shown that Pb exposure at 10 mg Pb^{2+}/mL of blood Pb to chromosomal aberrations in some aquatic organisms. Lead exposure in water (50 µg/L) over four weeks resulted in DNA strand breakage in the freshwater mussel *Anodonta grandis*. More recently, similar results (increase in the frequency of chromosomal aberrations and DNA damage

in kidney cell cultures) were observed in fish (*Hoplias malabaricus*) fed Pb-contaminated food over 18, 41, and 64 days (see AX7.2.3.4).

Environmental factors can alter the availability, uptake and toxicity of Pb to aquatic organisms. A study of the influence of abiotic variables, including dissolved organic carbon (DOC) on Pb concentrations in freshwater isopods found that, as DOC concentrations increased, BCFs decreased in *P. meridianus* and *A. aquaticus*, indicating that DOC acts to inhibit the availability of Pb to these isopods. Schwartz et al. (2004) collected natural organic matter (NOM) from several aquatic sites across Canada and investigated the effects of NOM on Pb toxicity in rainbow trout (*Oncorhynchus mykiss*). The results showed that NOM in test water almost always increased LT_{50} (time to reach 50% mortality), and optically dark NOM tended to decrease Pb toxicity more than did optically light NOM in rainbow trout. Studies generally agree that the toxicity of Pb decreases as pH increases. As pH decreases, Pb becomes more soluble and more readily bioavailable to aquatic organisms (Weber, 1993). Acute and chronic toxicity of Pb increases with decreasing water hardness, as Pb becomes more soluble and bioavailable to aquatic organisms (Horne and Dunson, 1995c; Borgmann et al., 2005). There is some evidence that water hardness and pH work together to increase or decrease Pb toxicity (see AX7.2.3.4).

High Ca^{2+} concentrations have been shown to protect against the toxic effects of Pb. Ca^{2+} affects the permeability and integrity of cell membranes and intracellular contents. As Ca^{2+} concentrations decrease, the passive flux of ions (e.g., lead) and water increases. Finally, increasing salinity was found to decrease Pb toxicity. The reduction in toxicity was attributed to increased complexation of Pb^{2+} with Cl^{-} ions (see AX7.2.3.4).

Also, nutrients (e.g., nitrate, carbonate) have been shown to affect Pb toxicity in some aquatic organisms. A study of blue-green algae (*Synechococcus aeruginosus*) exposed to 200 mg Pb/L indicated that additional nitrogen, phosphates, and some carbon sources (including sodium acetate, citric acid and sodium carbonate) all protected the algae from Pb toxicity at 200 mg Pb/L. The protective mechanism is still not clear. One hypothesis was that nutrients were able to reverse toxic effects. The second hypothesis was that nutrients directly interacted with Pb, in some way sequestering the metal so as to inhibit its metabolic interaction with the organism (see AX7.2.3.4).

Interactions with Other Pollutants

Predicting the response of organisms to mixtures of chemicals is a daunting task. There are two major approaches to predict mixture toxicity including: 1) examining the combined mode of action of the individual mixture substances; and 2) determining whether an organism response to the mixture is additive, or some deviation from additive (synergistic or antagonistic). In addition, researchers may report mixture toxicity in terms of additive concentrations or additive effects, which can cause confusion in the interpretation of study results. For the studies presented in Section AX7.2.3.4, the authors primarily report mixture toxicity in terms of additive concentrations (i.e., the sum of the concentrations of each individual chemical in the mixture will result in a level of effect similar to the simple sum of the effects observed if each chemical were applied separately).

When two or more metals compete for the same binding sites or interfere with transport through cell walls or membranes, the interaction is termed less than strictly additive or antagonistic. Antagonistic interactions can reduce metal bioavailability when metals are present in combination, and may lead to reduced potential for toxicity. There are a number of elements (Ca^{2+}, Cd^{2+}, Mg^{2+}, Na^{+} and Cl^{-}) that act in an antagonistic fashion with Pb (see AX7.2.3.4). For example, Pb is a well-known antagonist to Ca^{2+}, an essential element required for many physiological processes in most organisms.

Synergism occurs when the interaction of two or more metals causes an effect that is greater than the effect observed from the individual metals themselves. Synergism is likely the result of increased bioavailability of one or more of the metal ions due to the presence of other metals. Synergistic interactions have been observed with Pb and other metals (Cd, Cu, Ni, Zn) (see discussion in AX7.2.3.4).

The two most commonly reported Pb-element interactions are between Pb and calcium and Pb and zinc. Both calcium and zinc are essential elements in organisms, and the interaction of Pb with these ions can lead to adverse effects both by increased Pb uptake and by a decrease in Ca and Zn required for normal metabolic functions.

Effects of Lead on Primary Producers

Several studies have been conducted since the 1986 Lead AQCD on the toxicity of Pb to primary producers. Effects on algal growth (*Chlorella vulgaris*, *Closterium acerosum*,

Pediastrum simplex, *Scenedesmus quadricauda*), ranging from minimal to complete inhibition, have been reported at Pb concentrations between 100 and 200,000 µg/L. The toxicity of Pb to aquatic plant growth has been studied using *Spirodela polyrhiza*, *Azolla pinnata*, and *Lemna gibba*. Test durations ranged from 4 to 25 days and test concentrations ranged between 49.7 and 500,000 µg/L. Research on aquatic plants has been focused on Pb effects on aquatic plant growth, chlorophyll and protein content (see AX7.2.4.2).

Algae and other aquatic plants have a wide range in sensitivity to the effects of Pb in water. Both groups of primary producers experience EC_{50} values for growth inhibition between ~1,000 and >100,000 µg/L (see AX7.2.4.2). Exposure to Pb in combination with other metals generally inhibits growth less than exposure to Pb alone. Studies have shown that Pb adversely affects the metabolic processes of nitrate uptake, nitrogen fixation, ammonium uptake, and carbon fixation. Lead in combination with nickel or chromium produced synergistic effects for nitrate uptake, nitrogenase activities, ammonium uptake, and carbon fixation.

Effects of Lead on Consumers

The 1986 Lead AQCD (U.S. Environmental Protection Agency, 1986a) reported that hematological and neurological responses are the most commonly reported Pb effects on aquatic vertebrates. These effects include red blood cell destruction and inhibition of the enzyme ALAD, required for hemoglobin synthesis. The lowest reported exposure concentration causing either hematological or neurological effects was 8 µg Pb/L (U.S. Environmental Protection Agency, 1986a).

More recent literature on the toxicity of lead to fish and aquatic invertebrates has been summarized by Eisler (2000). Exposure of invertebrates to Pb can lead to adverse effects on reproduction, growth, survival, and metabolism. Water-borne Pb is highly toxic to aquatic organisms, with toxicity varying, depending on the species and life stage tested, duration of exposure, the form of Pb tested, and water quality characteristics (see AX7.2.4.1). Among the species tested, aquatic invertebrates, such as amphipods and water fleas, were the most sensitive to Pb effects, with adverse effects being reported at water Pb concentrations as low as 0.45 µg/L (range: 0.45 to 8000 µg/L). Freshwater fish demonstrated adverse effects at concentrations ranging from 10 to >5400 µg/L, generally depending upon water quality parameters (e.g., pH, hardness, salinity). Amphibians tend to be relatively tolerant of Pb, but may exhibit decreased

enzyme activity (e.g., ALAD reduction) and changes in behavior (e.g., hypoxia response behavior). Lead tends to be more toxic in longer-term exposures, with chronic toxicity thresholds for reproduction in water fleas ranging as low as 30 µg/L.

Effects of Lead on Natural Aquatic Ecosystems

Lead exposure may adversely affect organisms at different levels of organization, i.e., individual organisms, populations, communities, or ecosystems. Generally, however, there is insufficient information available for single materials in controlled studies to permit evaluation of specific impacts on higher levels of organization (beyond the individual organism). Potential effects at the population level or higher are, of necessity, extrapolated from individual level studies. Available population, community, or ecosystem level studies are typically conducted at sites that have been contaminated or adversely affected by multiple stressors (several chemicals alone or combined with physical or biological stressors). Therefore, the best documented links between lead and effects on the environment are with effects on individual organisms.

Recent studies on exposure to Pb in laboratory studies and simulated ecosystems indicate that Pb may alter species competitive behaviors, predator-prey interactions, and contaminant avoidance behaviors. Alteration of these interactions may have negative effects on species abundance and community structure (see AX7.2.5.2). For example, reduced avoidance behaviors have been observed at Pb concentrations ranging from 0.3 to 1.0 mg/L. The feeding behaviors of competitive species in some aquatic organisms are also influenced by the presence of Pb. Lead (6 to 80 mg/L) has also been found to reduce primary productivity and increase respiration in an algal community; and laboratory microcosm studies found reduced species abundance and diversity in protozoan communities exposed to 0.02 to 1 mg Pb/L. Lastly, numerous field studies have associated the presence or bioaccumulation of Pb with reductions in species abundance, richness, or diversity, particularly in benthic macroinvertebrate communities. In natural aquatic ecosystems, Pb is often found coexisting with other metals or other stressors. Thus, understanding the effects of Pb in natural systems is challenging given that observed effects may be due to cumulative toxicity from multiple stressors.

The effects of Pb have primarily been studied in relation to point source pollution rather than area-wide atmospheric deposition. Thus, the effects of atmospheric Pb on aquatic ecological condition remain to be defined. There is a paucity of data in the general literature that

explores Pb effects in conjunction with all or several of the various components of ecological condition as defined by the EPA (Young and Sanzone, 2002). However, numerous studies are available that associate the presence of Pb with effects on biotic conditions.

8.7.3 Application of Critical Loads to Terrestrial and Aquatic Ecosystems

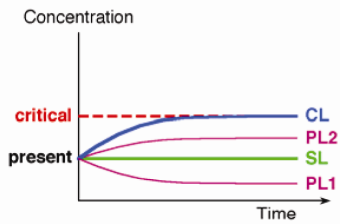
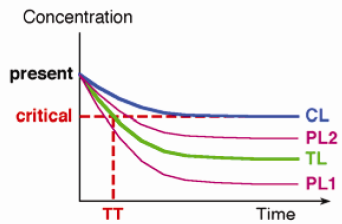
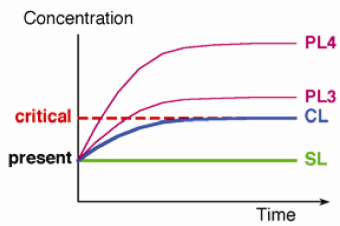
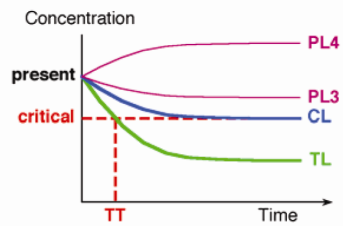
For the purpose of this section, critical loads are defined as threshold deposition rates of air pollutant that current knowledge indicates will not cause long-term adverse effects to ecosystem structure and function (see Section 8.3.1). A combinatorial application of critical limit and critical load allows one to assess current risk while simultaneously estimating future risk from exposure to a chemical. Figure 8-13 shows that four combinations of critical load and limit exceedance or non-exceedance are possible for a given ecosystem (Figure 1 of De Vries et al. [2004]). For example, if a current risk is indicated by an exceedance of the critical limit for Pb due to historical Pb deposition, but current inputs of Pb to the ecosystem are below the critical load (upper right corner), the critical load model predicts that Pb concentrations will fall below the critical limit at some point in the future if Pb deposition is maintained at the present level. If current soil concentrations are below the critical limit (lower left corner), inputs greater than the critical load will not result in exceedance of the critical limit for some period of time, but continued exceedance of a critical load will eventually lead to an exceedance of the critical limit. The time until a critical limit is exceeded (critical time) can also be predicted using the critical load model. This requires knowledge of current concentrations, the critical load, and predicted deposition rates. Critical times may be useful for setting priorities between ecosystems with critical load exceedances or between different chemicals.

Calculation of Critical Loads

This section summarizes various methods used to calculate critical loads (De Vries et al., 2001, 2002, 2004; Groenenberg et al., 2002), with an emphasis on the most recent material.

Critical Limits

To determine the critical limit, effects-based criteria for the major ecological endpoints should be developed for the ecosystem of concern. Criteria may be developed for any receptor that is exposed to the chemical of concern deposited in the ecosystem. In terrestrial ecosystems,

	No critical limit exceedance	Critical limit exceedance
No critical load exceedance	***No damage at present or foreseen:***  ➔ Keep the Present Load (more stringent than Critical Load)	***Present damage but recovery in progress:***  ➔ Keep the Present Load (more stringent than Critical Load) or ➔ Consider Target Load to reach the critical limit in a defined time period (more stringent than Critical Load)
Critical load exceedance	***Future damage foreseen:***  ➔ Consider Critical Load (emissions must decrease, even if concentrations in the ecosystem are allowed to increase further at critical load)	***Present damage, no recovery foreseen:***  ➔ Consider Critical Load (decrease of concentrations in the ecosystem down to critical limit in the long term) or ➔ Consider Target Load to reach the critical limit in a defined time period (more stringent than Critical Load)

CL - Critical load; PL - present load (2 cases); SL - Stand-still load; TL - Target load; TT - Target time

Figure 8-13. The predicted development of metal concentrations in ecosystems for four cases of exceedance or non-exceedance of critical limits and critical loads of heavy metals, respectively.

Source: Taken from DeVries et al. (2004).

possible ecological endpoints include effects from direct contact of invertebrates or plants with soil and ingestion of plants by herbivores. Effects-based criteria for use in defining the critical limit should be derived from ecotoxicological data appropriate to the most sensitive endpoint (De Vries et al., 2004). Regardless of the selected endpoint, the critical limit should be defined as a concentration in the medium that receives the depositional load, typically soil in terrestrial ecosystems and surface water in aquatic ecosystems. To derive these values, uptake and/or food- chain modeling may be necessary.

Criteria for Pb vary widely and can be the largest source of uncertainty in a critical load calculation. One reason for the wide range in estimates of effects criteria is that Pb speciation is often not taken into account. This can result in variation in estimates of concentration for total Pb that is associated with adverse effects, since the fraction of Pb available to cause a toxic effect depends on chemical factors such as the pH or organic matter content. To develop effects-based criteria applicable to media with a pH or organic matter content different from the test medium, it is more appropriate to develop criteria based on the free concentration of Pb rather than the total Pb concentration.

Models

Critical loads for heavy metals are typically calculated using a steady state model that ignores internal metal cycling and keeps the calculations as simple as possible (De Vries et al., 2004). The critical load is equal to the atmospheric input flux, which equals the sum of the output fluxes from the system minus the other input fluxes (e.g., weathering) when the concentration of Pb is at the critical limit. The input flux of heavy metals via weathering is sometimes neglected, because quantitative estimates are highly uncertain, and weathering is generally thought to be a relatively minor process (see Section 7.3.4.2).

Critical Loads in Terrestrial Ecosystems

Critical loads for Pb have been calculated using simple mass balance, dynamic, and probabilistic models for forested and agricultural land in Europe and Canada in a handful of preliminary studies. The methods and model assumptions used to calculate critical loads vary widely between these studies and little attempt has been made to validate the models that were used, so it is not known how much various simplifying assumptions affect the results.

In spite of the variation in methods and model assumptions used to calculate critical loads for Pb, some general conclusions may be drawn. The critical limit is the most important value for determining the value of the critical load. Wide variations in available effects levels makes this parameter one of the most important sources of uncertainty when calculating critical loads in terrestrial ecosystems. Spatial variations in critical loads for Pb are largely controlled by net runoff. Weathering and uptake by harvestable vegetation were less important. The time to reach steady state is several hundred years in the two studies that used dynamic models to determine critical loads.

Critical Loads in Aquatic Ecosystems

Doyle et al. (2003) modeled critical loads in surface water bodies assuming complete mixing with dilution water entering from the terrestrial catchment area. Loss of metal was also assumed to occur through downstream flushing and burial in sediment. Transfer of metal to sediment was modeled as a first-order process dependant on the dissolved concentration and pH. The inputs to the model included the following: water body area, terrestrial catchment area, water body depth, sediment accumulation rate, thickness of biologically active sediment, net precipitation, and water pH. The fist-order rate constant for transfer to sediment was correlated with pH. The model reached steady state within a few years. Transfer of Pb from the terrestrial catchment to the water body was neglected, because the time to steady state could be on the order of 10,000 years if the model included this source of Pb. However, the authors cited a separate calculation that indicated that neglect of transfer of Pb from the catchment may lead to a 5-fold underestimation of Pb concentrations in the surface water. These results indicate that Pb run-off from soil is more important than direct atmospheric deposition to the surface water bodies considered in this study. Due to the long times required to achieve steady state, the critical load methodology may not be appropriate for Pb in aquatic systems.

Limitations and Uncertainties

The largest sources of uncertainty identified in studies of critical loads for Pb include the following: (1) steady-state assumption; (2) derivation of the critical limit; (3) Pb speciation; and (4) soil runoff as an input to aquatic ecosystems

The critical load is calculated for steady state conditions, but the time for Pb to reach steady-state concentrations can be as long as several centuries. Thus, dynamic models are often used to predict Pb concentrations over shorter time frames. Dynamic modeling requires additional knowledge about current concentrations in the considered ecosystem. For regulatory purposes, use of dynamic modeling requires that a target time be set in order to calculate a critical load.

Speciation strongly influences the toxicity of Pb in soil and water and partitioning between dissolved and solid phases determines the concentration of Pb in soil drainage water, but it has not been taken into account in most of the critical load calculations for Pb performed to date. Recent guidance for heavy metals has begun to emphasize the importance of speciation to critical load calculations and suggest methods to calculate speciation (De Vries et al., 2004). To this end, Lofts et al. (2004) developed critical limit functions for several metals, including Pb, that take into account the effects of pH, organic matter, and the protective effects of cations on speciation.

Runoff of Pb from soil may be the major source of Pb into aquatic systems. However, little attempt has been made to include this source into critical load calculations for aquatic systems due to the complexity of including this source in the critical load models.

Preliminary efforts to calculate critical loads for Pb in terrestrial and aquatic ecosystems have so far relied on a variety of calculation methods and model assumptions. Efforts are ongoing to refine and standardize methods for the calculation of critical loads for heavy metals which are valid in the context of CLPTRP. At this time, the methods and models commonly used for the calculation of critical loads have not been validated for Pb. Many of the methods neglect the speciation of Pb when estimating critical limits, the uptake of Pb into plants, and the outflux of Pb in drainage water, limiting the utility of current models.

Future efforts should focus on fully incorporating the role of Pb speciation into critical load models, and validating the assumptions used by the models.

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